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FREEZE INDUCED TEXTURIZATION OF PROTEIN RECOVERED BY ALKALINE EXTRACTION FROM MECHANICALLY DEBONED POULTRY RESIDUES

by

© RICHARD A. LAWRENCE

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE
IN
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....
Supervisor

....

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Date........September 29/1981........

Abstract

The objectives of this research were a) to determine optimal conditions for protein recovery from mechanically deboned chicken residues, using low temperature alkaline extraction with subsequent acid precipitation, and b) to study the process of freeze induced texturization as applied to this protein concentrate.

Optimal protein recovery conditions were found with mild processing at 22°C, pH 10.5 and 30 minutes mixing time, with little improvement found with increasing severity of treatment. The mild conditions prevented the formation of new amino acids such as lysinoalanine, and limited the damage to the functional properties, which can occur under harsher conditions. The protein yield of the acid precipitated coagulum followed a general protein solubility curve with maximum recovery (85% of the extracted protein) and highest total solids (13 %) near pH 5.0. The precipitate had a protein concentration of approximately 75% (dry weight) and a fat concentration of 10%. The coagulum appeared independent of the acid used for precipitation.

Controlled freezing of this proteinaceous material with subsequent heat setting was found to produce a fibrous product, resembling meat, with generally parallel fibres, normal to the freezant/protein interface. Increasing the freezing rate (ambient temperature -5°C to -45°C) increased the fibre density, the extent of fibre crosslinking, and the water retention, and slightly decreased the peak force obtained using an Instron Universal Texture Testing Instrument with a Kramer Shear cell. Increasing the pH above the isoelectric point to pH 6.0, caused an increase in fibre density, fibre crosslinking and water retention, and a decrease in peak force. Increased initial total solids decreased the fibre density, while increasing the fibre size, the peak force and the final total solids . Analyses of the fibre structure using fibre density, peak force and final total solids,(or water retention), showed similar trends following changes in the process parameters,such as freezing rates, total solids and pH.

The uniformity of the fibre structure was improved by freezing in a semi-infinite cylindrical form. A semi-infinite slab form caused an increase in fibre size with increased distance from the freezant/protein interface. Binding agents were not required to bind the fibres together, due to the presence of fibre crosslinks. The product was relatively retort stable, with no loss of particles, although a time dependent reduction in peak force was evident, with up to a 35% reduction after retorting at 121°C for 60 minutes.

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Part I

Low Temperature Alkaline Extraction of Proteins from Mechanically Deboned Chicken Residue

1. Part 1: Alkali Extraction of Poultry Proteins

1.1 Introduction

Conversion of vegetable proteins into animal proteins such as poultry, cattle, or fish is an expensive and inefficient process. Comparing the protein yield to the required crude protein intake, the most efficient animal protein source, milk, is only 38% efficient, while other animal protein sources rapidly decrease in conversion efficiency (Table 1, Wilson, 1968).

Comparing the protein production to land usage, soybeans are vastly superior to animals by factors of 28 for beef, 17 for swine, 12 for poultry and 10 for milk (Catron, 1967). These data are somewhat misleading, in that much of the land used for animal production is of little use for the production of soybean or other high protein vegetables, and therefore can only be utilized as rangeland. However, it still remains that when man is a second order consumer, the cost of the protein, due to conversion inefficiencies, will be much higher than if he were a first order consumer, ie. vegetarian.

On the other hand, from a human nutrition standpoint, proteins from an animal source are of higher quality than those of a plant source, for they contain all the essential amino acids for a human diet (Mountney, 1976). Proteins from plant sources are usually deficient in one or more essential amino acids and therefore require a diet complement from

Table 1

Conversion Efficiency of Vegetable Proteins to Animal Proteins

Animal Protein Source	% Conversion Efficiency
Milk	38
Eggs	31
Poultry (broilers)	31
Fish	20
Pork	15
Lamb	9
Steer	6

(from Wilson, 1968)

Table 2

Protein Content of Cooked Meats

Meat	Protein Content (%)
Poultry	25 - 35
Beef	21 - 27
Pork	23 - 24
Lamb	21 - 24

(from Scott, 1956)

another protein source, either plant or animal, sufficient in those specific absent amino acids (Anglemier and Montgomery, 1976; Stillings, 1973).

Amongst the more regularly consumed animal proteins, poultry meat has one of the highest conversion efficiencies (Wilson, 1968) and in a cooked state, one of the highest protein contents (Table 2, Scott, 1956). The amino acid composition of poultry is very similar to beef and pork, with the limiting amino acids (with reference to a whole egg) being the sulphur amino acids (Scott, 1959).

Therefore, due to poor conversion efficiencies of animal protein production, high production costs, and high quality of animal proteins, it becomes essential that all usable high grade proteins, be recovered before rendering the waste material. Unfortunately, the process of slaughtering and processing any animal, poultry or fish, produces a loss of edible muscle protein due to its adhesion to the bone by-products. Hubbard (1972) found an average of 11 kg meat remaining on beef bones after typical boning out operations. Field (1976) suggests that this could amount to over 2,000,000 metric tons of meat from beef, pork and lamb sources in the U.S.A. annually. Although no specific data was found on poultry, the large amount of meat remaining on poultry necks and backs should also represent a high quantity of recoverable protein.

Subsequent rendering of this bone by-product to materials of a non-consumable nature for humans, ie. meat and bone meals for animal feed, fertilizers, tallows and greases, means a loss of profits and under-utilization of a high grade protein source. The present trend of increased centralized processing of the carcasses has produced a greater quantity of bone by-products in the processing plants rather than at individual stores or institutions. Within the poultry industry, this centralized processing, brought about by a consumer preference for precut poultry pieces, has produced a surplus of necks and backs which contain large amounts of poorly attainable but high grade protein. A similar situation exists in the beef industry where increased carcass breakdown to primal and sub-primal cuts to produce boxed beef is prevalent and causes an increase in bone by-products. This is further compounded by the increased use of hot boning, a process which leaves a greater percentage of muscle protein on the waste bone material (Taylor _et al_., 1980; Schmidt and Kerman, 1974; Cuthertson, 1980). As the meat on the bone wastes costs the same to produce as the more readily saleable cuts, it is to the advantage of the processors to recover as much of this protein as possible.

However, it must be remembered that it is not essential, nor is it in fact desirable, to remove all the protein from the bone wastes. Certain proteins, such as collagen, are of reduced nutritional and functional value for human grade products, as compared to sarcoplasmic and myofibrillar proteins (Swingler and Lawrie, 1979). These connective tissues should remain in the bone wastes to allow for the production of rendered products which can be utilized by other animals or as fertilizers. Absence of these proteins would drastically reduce the value of rendered products.

1.2 Research Objectives

Protein recovery from waste animal sources has been explored and used industrially for a number of years. However, mechanical deboning, the major recovery process presently being utilized, still leaves a waste product with usable high grade proteins. Furthermore, the use of mechanical deboners, especially in the poultry industry, has increased significantly in the last decade, producing a large supply of potentially recoverable protein.

With few exceptions, the alternate protein recovery methods, as reviewed in the next section, are either not suitable for the production of human grade protein, or are utilizing relatively high concentration protein sources, usually from beef or fish, or severe extraction environments, (ie. high temperature, high pH, long retention time). The major aim of the first part of this work was to optimize the recovery of protein from a mechanically deboned poultry residue source using a low temperature, alkaline extraction process. Effects of different food grade acids and pH of acid precipitation on protein recovery and the protein loss to the supernatant whey was investigated.

- Effects of the extraction environment, ie. temperature, time, and pH on the formation of new amino acids, particularly lysinoalanine, was also examined.

2. Literature Review

2.1 Methods of Protein Recovery from Waste Meat By-Products

2.1.1 Mechanical Deboning

Mechanical deboning was introduced in the late 1940's for the separation of meat from otherwise unconsumable or unpopular fish (Anon, 1979). Mechanical deboning of poultry began ten to fifteen years later as the preference for precut chicken pieces increased and as a market grew for specialty items which could be produced from the mechanically deboned meat product. Mechanical deboning of beef started a few years later. Over two hundred million pounds of mechanically deboned poultry meat (MDPM) were processed in the United States in 1977 (Anon, 1979) with increases expected each subsequent year. U.S.D.A. estimated that over one million tons of red meat (beef, pork, lamb) was produced by mechanical deboning in 1977.

A number of different mechanical deboning processes exist, with new patents emerging frequently. These differ in the yields of protein (sarcoplasmic, myofibrillar and collagen), fats, and varying bone content and particle size. The basic process takes the bone and/or carcasses (as in poultry), coarsely crushes the bones, causing separation of meat from bone, and in some manner subsequently removes the meat from the bone. This usually occurs by forcing this material against a screen or slotted surface, which forces the majority of the edible protein and fat in a paste form through, while the large bone particles remain behind. Machines presently in use vary in the methods by which the bone is crushed, how it is forced through the screen and the design of the screen. Various deboning processes include the Beehive, Paoli, K.P. Bone Removal System, Bibun, Prince, Protecon, and Sepramatic Systems (Froning, 1976; Anon, 1976; Katz and Ackroyd, 1979; Protein Foods, 1980) with recent patents by Van Rij and Smits, (1979) and Van Bergen (1979).

Yields are dependent on the material being deboned, the type of machine used and the machine settings, which directly affect the yield and quality of the product, i.e. fat, protein (collagen) and bone content. Yields of up to 92% from whole fish and 43% from pork loin bones were reported for the Paoli system(Anon, 1976).

Compositional standards for mechanically deboned meat have been set by the U.S.D.A., limiting the amounts of fat, skin, bones and various chemicals, along with a minimum protein content, depending on the final use of M.D.M. Canadian regulations specifically control bone content, with general guidelines following the U.S. standards. However, the actual composition of the M.D.M. has been shown to vary significantly (Froning and Johnson, 1973; Froning, 1970; Froning, 1979; Grunden _et al_., 1972; Goodwin _et al_.,1968; Satterlee, 1975). The major causes for variability are due to the differences in starting materials, ie. bone to meat ratio, fat content, skin content, age, cutting methods, machine setting (ie. hole size) and subsequent separation steps, (ie. centrifugation) (Froning, 1976; Froning and Johnson, 1973; Protein Foods, 1980).

2.1.2 Mechanically Deboned Poultry

Yields from whole poultry carcasses are usually in the 60-70% range (Paoli, Beehive) though the Sepramatic process used by Protein Foods Ltd. has produced a consistent yield of over 80% (Protein Foods, 1980). Amino acid analysis of mechanically deboned poultry meat (MDPM) was shown to be similar to hand deboned products (Essary and Ritchey, 1968) providing a high grade protein source. However, proximate analyses of MDPM from various materials have shown a relatively high fat content (Table 3, Froning, 1976).

Increasing the skin content of the initial material was shown to increase the fat content, while decreasing the moisture, protein, and calcium content. In most units, increasing the skin content did not increase the collagen content of the protein in the paste, as the skin is retained by the screen with the waste bone residue (Satterlee _et al_., 1971). However, the Sepramatic process has been shown to allow a greater content of skin to pass through, thereby increasing the collagen content (Protein Foods, 1980). High fat and skin concentrations in the MDPM have been found to impair the emulsifying capacity of the MDPM, though the high skin content has been shown to increase binding capabilities (Maurer and Baker, 1966; Protein Foods, 1980). Reduction of the fat prior to or after deboning increases the emulsifying

Table 3

Proximate Data of Mechanically Deboned Poultry Meat

Mechanically Deboned Material	Protein (%)	Moisture (%)	Total Lipids (%)
Chicken backs & necks	14.5	66.6	17.6
Chicken backs & necks	9.3	63.4	27.2
Chicken backs	13.2	62.8	21.2
Turkey frame meat	12.8	70.7	14.4
Turkey frame meat	12.8	73.7	12.7
Spent layers	14.2	60.1	26.2
Spent layers	13.9	65.1	18.3

(from Froning, 1976)

capacity (Froning and Johnson, 1973; Goodwin et al., 1968). Increasing the salt soluble proteins (sarcoplasmic and myofibrillar), as compared to connective tissues proteins, increased the emulsifying capacity (Hudspeth and May, 1967; Froning, 1970) along with increasing the nutritional quality of the product (Chang and Field, 1977). The emulsifying capacity and water holding capacity was also improved by the addition of polyphosphates and sodium chloride (Froning, 1966; Froning and Johnson, 1973; Schlamb and Bortsky, 1971).

2.1.3 Possible Risks of Mechanically Deboned Meat

The nature of this process requires relatively tight controls to prevent possible hazards in the product. In the vast majority of the deboners, the bones are extensively crushed to enable meat separation. The Protecon system varies slightly with a reduced bone fragmentation (Katz and Ackroyd, 1979). In other cases, bone fragments, which can be as large as the size of the slots, screen holes or slits can pass through with the MDM. Work by Froning (1979) showed deboned turkey to have an average bone particle size of 233 μ with a maximum up to 536 μ (screen hole size). The concern over bone chip size is ill deserved, as bone particles from hand deboned turkey have been found up to 1142 μ. The bone pieces are so small as to be individually undetectable when consumed. It was also shown that these particles readily dissolved in an acid concentration similar to those in human gastric juices, therefore suggesting these bones to be a good calcium source rather than a health risk (Field et al., 1977). A U.S.D.A. study of this problem has concluded that these bones do not constitute a hazard to humans, assuming they remain within regulation limits of .75% residual calcium from bones (Kolbye et al., 1977).

The possibile presence or accumulation of trace elements was studied by a U.S.D.A. committee (Kolbye et al., 1977) and it was concluded that for red meats the long term use of MDM would not pose hazards, nor be different from the effects of other processed meats. It was suggested that a difference could exist with poultry bones from culled laying hens which may exhibit an accumulation of fluoride (Anon, 1979). Klose (1979) showed a concentration between 2-5 ppm fluoride in MDPM, with a ratio of 1:13 fluoride content between the MDPM and the bone residue. He suggests the only possible health risk may exist with infants and therefore MDPM should not be used in infant food formulations.

Microbial contamination may be the greatest health hazard with MDM, as its paste-like nature can be an ideal growth medium for microorganisms. In comparison to conventionally processed meats, MDPM held 5 days at 3-5°C before deboning exhibited higher total aerobic counts. The total aerobic counts increased during storage at 3°C, while fecal coliforms were high, though remained constant throughtout storage. Six of 54 samples were contaminated with Salmonella, four showed presence of Clostridium perfringens, but there was no sign of Staphylococcus aureus.

The psychrotolerant genera isolated were dominated by Pseudomonas, Achromobacter and Flavobacterium. Microbial counts for MDPM were found to range between 10^5 to 10^6/gm, with coliform counts between 10^1-10^3/gm (Maxcy et al., 1973).

2.1.4 Bone Residues from Mechanical Deboning

Mechanical deboning does not remove all of the protein from the boning wastes. The twenty to forty percent of the raw material which is retained by the deboner is usually rendered. The protein concentration and content of this mechanically deboned residue will be dependent on the deboning process. Young (1976) found the poultry bone residue from a Mark IV Princeworld deboner to contain 40% solids, of which 43% was protein, 32% fat, 25% ash. A high percentage of the protein is likely to be collagen due to the high skin content. However, high grade proteins are also retained and these could and should be better utilized. Young (1976) suggested that a protein isolate amounting to 2-3% of the total residue or 12 to 18% of the proteins can be recovered from mechanically deboned bone residue by salt extraction. Various processes, including alkaline and salt extractions are suitable for such protein recovery.

2.2 Alkaline Extraction

Alkaline treatment of foods and food proteins has been used within North America for centuries, dating back to alkaline treatment of corn or maize by central American Indians (Katz *et al*., 1974). More recently, alkaline treatment has been used extensively for extraction and solubilization of proteins from both meat and vegetable sources. Industrially, only vegetable protein concentrates and isolates, produced by alkaline extraction or solubilization, have been used for subsequent texturization processes. Most research has dealt primarily with alkaline extraction with subsequent acid precipitation, though the effects of acid extraction have been examined. Lampitt *et al*. (1952, 1954) showed that dilute NaOH is more effective than dilute HCl in extracting muscle tissue from connective tissue, as the dissolution was more complete and less collagen was dissolved. The latter was also shown by Swingler and Lawrie (1979). Miyada and Tappel (1956) found dilute NaOH to be more effective than either dilute potassium chloride (Duerr and Earle, 1974) or urea. Young (1976) showed alkaline extraction to be slightly more effective than extraction with a high ionic strength NaCl solution. Meinke *et al*. (1972) also showed a similar difference between alkaline and salt extraction using fish proteins.

Processes for alkaline extraction of proteins have been investigated and used on industrial and lab scales. Although soybean is the primary vegetable source for industrial protein extraction/solubilization, rapeseed and other oilseeds have also been studied (UNIDO, 1974; Hermansson et al., 1974; Chamnanwej, 1971; Shaikh et al., 1968; Yeyha, 1981). Alkaline extraction/solubilization of most animal sources have dealt primarily with by-products or products of an initially non-palatable nature which would subsequently be used inefficiently. Alkaline solubilization of water insoluble protein concentrates has been researched on heat precipitated cheese whey protein (Jelen and Schmidt, 1976), fish protein concentrate (Tannenbaum et al., 1970a, 1970b) and rapeseed protein concentrate (Hermansson et al., 1974).

The effect of various parameters on alkaline extraction and subsequent acid precipitation has been studied with different animal offal sources, though little has been done on poultry, or the residue from the mechanical deboning process. Of primary concern are the effects of extraction pH, temperature, retention time, solute/solvent ratio, salt additives and protein sources.

2.2.1 Protein Source

The solubility of proteins versus pH follows a general curve as shown in Fig 1. (Meinke et al., 1972). However, the percentage of total proteins extractable was found to be dependent on the initial material (Connell, 1964; Meinke et al., 1972; Swingler and Lawrie, 1979; Young, 1976; Young and Lawrie 1974). Meinke et al., (1972) and Connell (1964) found

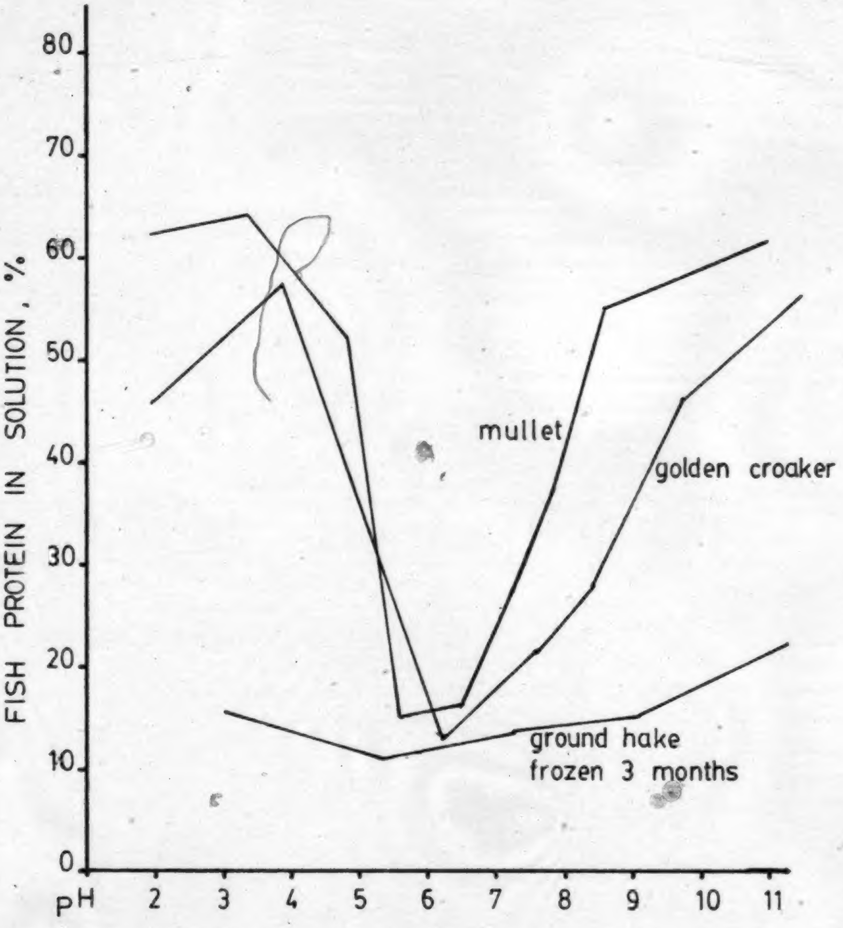

Figure 1 Solubility of Protein from Mullet Golden Croaker and Ground Hake Frozen Three Months.

(from Meinke et al., 1972)

variable solubilization from different fish sources (Fig. 1, Meinke *et al*., 1972). Swingler and Lawrie,(1979), and Young and Lawrie,(1974) showed a similar variation between types of bovine offal. Swingler and Lawrie (1979) suggest this may be partially due to larger concentrations of non-protein nitrogeneous compounds in certain sources. Frozen storage of certain species of fish has been shown to be detrimental to the solubility of the proteins (Fig.1) though other species have shown little effect of frozen storage (Meinke *et al*., 1972; Connell, 1964). Connell (1964) has indicated that it is the myofibrillar proteins (actomyosin), which are subject to the greatest protein denaturation while the solubility of the sarcoplasmic proteins are not affected by freezing.

2.2.2 Effect of pH

In solutions with no salt additives, the solubility of proteins reaches a minimum between pH 5 and pH 6, depending on the protein source. This relates to the general iso-electric point of meat and soy protein (Forrest *et al*., 1975). The rate of increase of protein solubility with increased alkalinity, along with the point of maximum solubility, depends primarily on the protein source, though in most cases extraction above pH 11 did little to increase the yield and also required large amounts of alkali (beef: Hamilton, 1978; Jelen *et al*., 1979a; fish: Meinke *et al*., 1972; Connell, 1964).

Swingler and Lawrie (1979) showed that the collagen content increased four fold by increasing the pH from 10 to 11, reducing the nutritional quality of the protein. Most researchers used an extraction pH between 10 to 11 which was found to give optimum protein extractability (Jelen _et_ _al_., 1978; Hamilton, 1978; Swingler and Lawrie, 1979; Meinke _et_ _al_., 1972).

The product extracted from beef was noted to have a strong fishy odor above pH 9.5 (Jelen _et_ _al_., 1979; Hamilton, 1978). The viscosity of the solution was found to increase substantially between pH 8-9.5, which hindered subsequent separation of the extract from fat and bones (Jelen _et_ _al_., 1979). S.D.S. gel electrophoresis showed that below pH 8.5 the extract contained primarily sarcoplasmic proteins (Hamilton, 1978).

2.2.3 Temperature

Hamilton (1978) found that the temperature had little effect on the yield of beef protein from extraction, and that the temperature choice was more dependent on microbiological considerations, equipment and an appreciation of undesirable secondary reactions (such as protein hydrolysis and new amino acid formation). Marginal increases in yield with beef were suggested by Jelen _et_ _al_ (1979a) at very low temperatures though extraction at room temperature, compared to 7 and 10°C, was preferable as it allowed for more efficient removal of fat from the extract.

In extracting water soluble proteins with water, Hamm and Deatherage (1960) found a maximum yield at temperatures below 20°C. At 60°C the yield is reduced to 23% of that at 20°C. The collagen content was also found to increase as the temperature increased above 50°C (Duerr and Earle, 1974). However, with alkaline extraction of beef proteins, Duerr and Earle found an increase in soluble proteins with increased temperature, along with a 150% increase in collagen content (60°C - 90°C). Unfortunately, extractions were not run below 60°C, even though the most dramatic changes in meat protein properties occur between 50 - 60°C. Extracting bovine offal at pH 10.0, at 0, 20, 40, and 60°C, Swingler and Lawrie (1979) found significant increases in protein recovery with increased temperature, with the recovery at 60°C being approximately twice that at 0°C. Again, an increase in collagen was noted. New amino acids were also found in significant quantities at 60°C, compared to traces at 40 and 20°C (see further discussion on page 28). Meinke et al. (1972) also noted an increase of 18-29% yield with an increase in temperature from 22°C to 55°C with fish protein.

2.2.4 Retention Time

Young and Lawrie (1974), found a maximum protein extraction of bovine and ovine offal at 0°C and 8 hours. Subsequent work by Swingler and Lawrie (1979) showed comparable recoveries at 20°C for 2 hours. However, research on beef boning room wastes show little increase in yield after 30 minutes of extraction (Jelen et al., 1979a; Jelen et al., 1978; Hamilton, 1978). Meinke et al. (1972) used an extraction time of 30 minutes with fish proteins.

2.2.5 Meat/Solvent Ratio

Extraction with a low meat/solvent ratio will produce a large quantity of liquid extract with a low protein content, whereas a high meat/solvent ratio produces a product with a low yield of viscous extract with a high protein content. The first case produces problems with effluent control and subsequent protein concentration, whereas the latter increases the difficulty of separation due to high viscosity, causing loss of protein (Jelen et al., 1979a; Hamilton, 1978).

In varying the ratio from 1:20 to 3:10, Meinke et al (1972) found the percentage of soluble fish protein in the solution remained essentially the same at 65%. However, when the ratio was varied at pH 11, the recoverable volume of extract dropped from 95% to 68%, thereby reducing the recoverable protein by 1/3. Hamilton (1978) compared the effect of meat/solvent ratio on the % beef protein in extract, yield of extract and available protein extracted (Fig. 2). Optimum extractability appears to be between 1:1 and 1:1.5, assuming the viscosity of the solution is not too difficult to handle. Ratios of 1:1.25 and 1:1.5 were used by Jelen et al. (1979a) for beef bones.

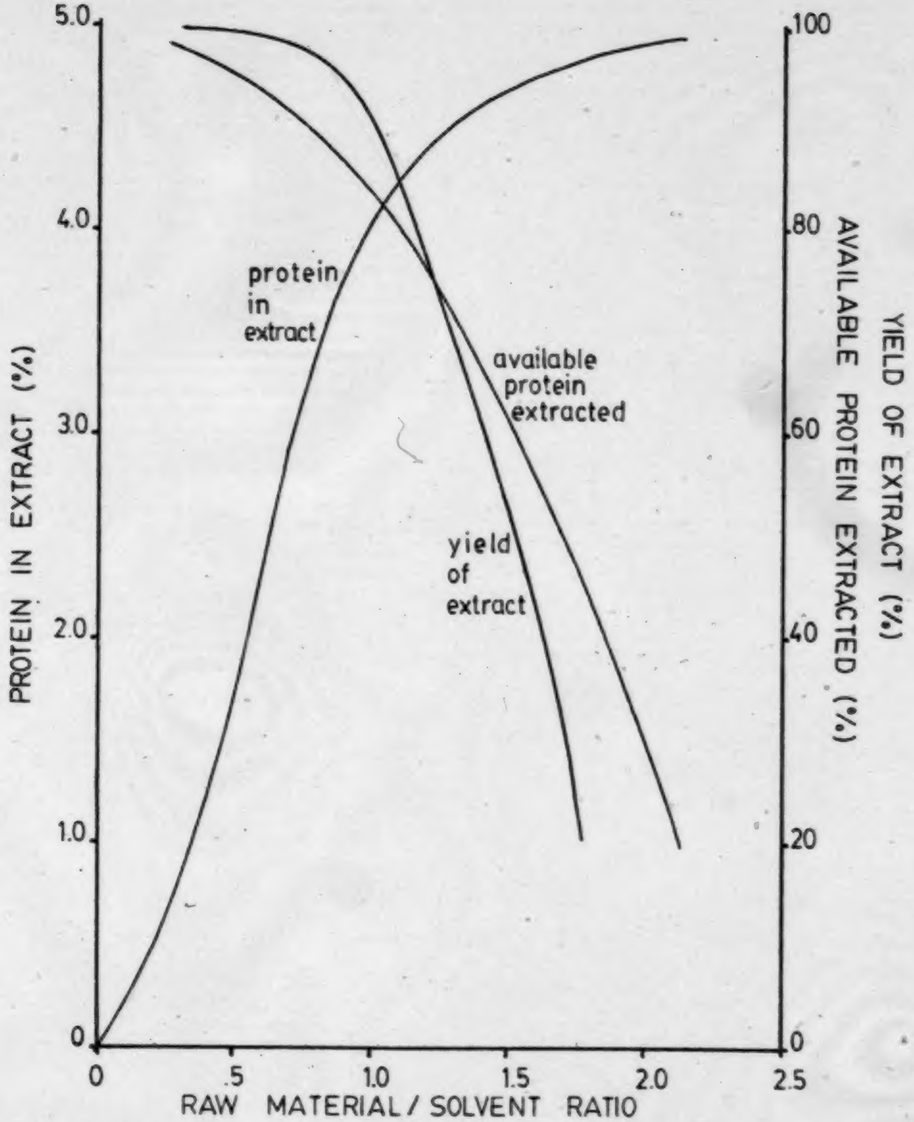

Figure 2 Effect of Raw Material to Solvent Ratio on the Protein Yield from Beef Trimmings (pH 11, 1 hour, 10° C).

(from Hamilton, 1978)

2.2.6 Extractant Additives

Increasing the ionic strength through addition of NaCl to the alkaline solution, showed a decrease in protein extracted at high and low pH values, and an increase in protein solubility at the point of minimum solubility near pH 6 (Meinke *et al*.,1972). The change produced a general shift in the iso-electric curve towards the acidic side, as expected from increased ionic strength effects (Pederson, 1971). At high alkaline conditions (pH 11), the addition of extractants (NaCl, tetrasodium pyrophosphate, NaCl + TSPP) reduced the protein solubility (Jelen *et al*., 1979a).

2.2.7 Precipitation of Proteins

Iso-electric precipitation of the proteins through the addition of acid is the most common method of concentrating or precipitating the proteins of soybean and other oilseeds (UNIDO, 1974), fish (Kahn *et al*., 1975; Meinke *et al*., 1972), poultry (Young, 1976) and beef offal (Swingler & Lawrie, 1979; Young, 1980). In most cases, maximum precipitation occurs between pH 4.5 - pH 6, leaving between 14% and 30% of the protein in solution (Meinke *et al*., 1972; Swingler and Lawrie, 1979). This can be accounted for by the water soluble sarcoplasmic protein fraction, which amounts for up to 40% of the protein (Hamilton, 1978). Unless the acid is added to a rapidly agitated solution, preferably in a diluted form, problems may arise from acid precipitation due to pockets of extreme acid pH which cause protein denaturation and damage (Hamilton, 1978).

Other methods of concentration were investigated by Jelen et al., (1978) and Hamilton, (1979). Ultrafiltration concentration of proteins was suggested, though dismissed due to protein clogging of the filtration membrane, along with cost deterents (Hamilton, 1978). Jelen et al. (1978, 1979a) investigated the effect of heat on the acidified slurry. The highest protein recovery was found with boiling at pH 6.0, or less, though the heat denatured material was gritty and incohesive, and lacked most of the desirable functional characteristics. The heat coagulability of sarcoplasmic proteins would account for the increase in protein recovery found at elevated temperatures (Hamm, 1970). In general, Jelen et al. (1979a) found an increase of 10% protein precipitated with severe heat treatments, as compared to acid precipitaton at room temperature or intermediate heating at 60°C. As heat denaturation of protein occurs between 50 - 60°C (Duerr and Earle, 1974), some coagulation is expected to occur at these temperatures, reducing the functionality of the protein.

Protein recovery by freezing was tried by both Jelen et al. (1979a) and Hamilton (1978). Jelen found the freeze thaw effect to slightly reduce the protein recovery as compared to acidification at room temperature. Freeze separation, which involved separating the frozen water from the concentrate by filtration proved difficult due to an increased viscosity caused by an increased concentration, which subsequently made ice separation a problem (Hamilton, 1978). Although this method requires less energy than evaporation, it is still energy intensive.

2.2.8 Sensory Evaluation

Sensory evaluation was carried out on alkali extracted beef protein concentrate alone (Jelen et al., 1979a) and as a replacement for a portion of the meat in comminuted products (Jelen et al., 1979b; Hamilton, 1978). Alone, the concentrates were found to be "bland, lacking the characteristic meaty flavors, and relatively free of objectional off flavors," (Jelen et al., 1979a). The acid and freeze thaw precipitated proteins were found to have better textural qualities than the acid-heat precipitated proteins.

Incorporating the concentrate into luncheon meats showed; a) no significant color change with up to 20% concentrate, b) a slight decrease in Kramer Shear cell peak force texture measurements with increased concentrate, with a maximum suggested level of 15%; c) a threshold value of 15% for flavor and overall quality (Jelen et al., 1979b). However, it was suggested that this threshold level may increase with appropriate formulation adjustments. Hamilton (1978) found that at 50% replacement a slightly greater weight loss due to cooking (.6 to 1.6%) occurred, along with a slight decrease in gel strength (1.21 to .98 kgf), while the material did not change in color.

2.2.9 Chemical and Nutritional Modifications Due to Alkaline Processing

The extent of amino acid modifications due to alkaline processing is heavily dependent on the severity of the process, i.e. pH, temperature, and retention time. Modifications of primary concenn are the formation of toxic or non-metabolizable amino acids and the reduction of essential amino acids.

Severe alkali treatments of proteins are known to cause destruction of certain amino acids, i.e. lysine, cystine, arginine, threonine and serine (Blackburn, 1968; Mellet, 1968; Provansal *et al*., 1975). Provansal *et al*. (1975), working on sunflower proteins, found significant losses occurring only during severe treatments (>55°C, >pH 11.5, 1 hr), while only cystine was affected by milder conditions. Under essentially alkaline hydrolysis conditions (pH 12.9, 80°C, 16 hrs) arginine and cystine were totally destroyed, with serine and threonine losses of 55% and 82%. De Groot and Slump (1969) carried out similar experiments with a shorter maximum retention time of 4 hours (Table 4). The majority of amino acids remained relatively constant or increased in concentration. Significant cystine destruction occurred above 40°C at pH 12.2. (70% destroyed at pH 12.2, 80°C), though time did not appear to be a factor. Lysine and serine reduction occurred only at high pH and temperature (pH 12.2, >60°C). Alkaline treatment of fish showed similar trends (Carpenter *et al*., 1952). As the majority of food

Table 4

Essential Amino Acids and Lysinoalanine Composition of Isolated Soy Protein after Various Treatments

Treatment												
pH	-	7	12.2	12.2	12.2	12.2	12.2	12.2	12.2	7	8	10
Temperature°C	-	23	40	40	40	40	23	60	80	40	40	40
Duration, hr.	-	0	1	2	4	8	4	4	4	4	4	4
Isoleucine	5.0	5.0	5.0	5.3	5.3	5.6	4.8	5.3	5.7	5.0	5.2	5.1
Leucine	7.5	7.7	7.8	8.3	8.4	8.7	8.1	9.1	9.7	8.1	8.0	7.9
Lysine	5.7	5.8	5.6	5.2	5.4	5.1	5.6	4.9	4.7	5.8	5.5	5.7
Phenylalanine	5.3	5.4	5.6	5.8	5.7	6.0	5.6	6.3	6.4	5.6	5.6	5.3
Methionine	1.25	1.15	1.16	1.17	1.16	1.23	1.17	1.25	1.39	1.26	1.26	1.27
Threonine	3.5	3.5	3.5	3.5	3.6	3.7	3.6	3.5	3.4	3.8	3.9	3.8
Tryptophan		1.1			1.1	1.15			1.3			
Valine	5.0	5.0	5.0	5.0	5.5	5.6	5.1	5.6	6.4	5.2	5.1	5.2
Lysinoalanine	0.0	0.0	0.42	0.68	0.83	1.09	0.40	1.71	2.08	0.0	0.0	0.10

(from De Groot and Slump, 1969)

proteins are limited by either sulphur containing amino acids or lysine, their destruction will reduce the proteins nutritive value (de Groot and Slump, 1969).

Under milder conditions, i.e. pH 11, and room temperature, Meinke et al., (1972) found an increase in all essential amino acids over fresh fish, except for tryptophan, whose disappearance could not be explained. However, Golan and Jelen (1979) found isoleucine, lysine, methionine, phenylalanine, and tryptophan to drop in concentration over fresh meat muscle, though they all exhibited an expected increase over the raw ground bone material.

The percentage of essential amino acids of the extracted fish protein concentrate was found to be higher than either fresh fish or isopropanol extracted concentrate (Meinke et al., 1972). Similarly, this was found with alkaline extracted beef protein concentrate which was higher than mechanically deboned beef (Golan and Jelen, 1979). This suggested a better removal of bone collagen, connective tissue and inorganic bone material.

Provansal et al., (1975) found that isomerization of L-lysine to D-lysine became significant at pH 11.5, 80°C, and one hour, with marked increases as severity increased. Similar results were shown to occur with cystine, methionine, alanine, tyrosine, phenylalanine, and aspartic and glutamic acid (Hill and Leach, 1964; Pollock and Frommhagen, 1968). Nutritionally, this is likely to reduce the availability of the proteins, as D-lysine and other D-amino acids are not biologically available to organisms (Berg, 1959).

2.2.10 Production of New Amino Acids

Several new amino acid residues have been found as a result of alkaline treatment of proteins. These were identified as lysinoalanine, lanthionine, ornithinoalanine, ornithine, and alloisoleucine (Aymard et al., 1978; Bohak, 1964; Freidman, 1977; Friedman, 1979; Finley and Friedman, 1977; Finley et al., 1978; Provansal et al., 1975; Slump, 1978; Sternberg et al., 1975a; Sternberg et al., 1975b; Zeigler et al., 1967). These amino acids appear to be derived from serine, cystine, lysine, histidine, arginine and possibly tryptophan (Finley et al., 1978; Finley and Friedman, 1977). The presence of lysinoalanine (LAL) has been reported to reduce protein digestibility and net protein utilization (de Groot and Slump, 1969) along with causing kidney damage in rats by renal lesions, characterized by nuclear and cytoplasmic enlargement in rats (Gould and MacGregor, 1977; O'Donovan, 1976; Woodard and Short, 1973). However, this nephrotoxic effect appeared to be species specific to rats; as mice, hamsters, Japanese quail, dogs and monkeys failed to exhibit these symptoms (Gould and MacGregor, 1977, Sternberg et al, 1975a). Although these alterations were noticed when rats were fed soy protein, subjected to alkali treatment (Woodard and

Short, 1973), other workers were unable to duplicate these results (van Beek et al., 1974) without feeding LAL as a free amino acid (de Groot et al., 1978). Finley and Friedman (1977) suggest that the poor correlation between LAL content of proteins and nephrotoxicity in rats may result in contributions from other crosslinked amino acids. However, the relative sensitivity of man to LAL or other new amino acids is unknown, as present data suggests a marked differential susceptibility amongst species to the induction of this lesion (Gould and MacGregor, 1977). Furthermore Gould and MacGregor (1977) suggest that "it is likely that a number of factors other than simple dietary LAL content determine the biological response to alkali-treated proteins".

Production of the new amino acids have been found to be dependent on the severity of the alkali heat treatment, i.e. pH, temperature, retention time and the protein source (Table 4) (de Groot and Slump, 1969; Sternberg et al., 1975a; Sternberg and Kim, 1977). With alkali-extracted meat protein products (pH 10, 2 hrs) Swingler and Lawrie (1979) found only trace amounts of LAL at 20 and 40°C with measurable amounts at 60°C (3900-4900 ppm). However, Sternberg et al. (1975a, 1975b) have found LAL in a number of cooked products at low alkaline conditions. Furthermore, they found LAL in heated products which were never subjected to an alkaline environment. LAL has also been found to form in fresh eggs after several days storage (pH 9.0) illustrating very minimal heat requirements (de Groot et al., 1978). As such then, humans have been exposed to proteinaceous material containing LAL since processing (i.e. cooking) was started. It is perhaps more important that the LAL content in proteinaceous materials becomes an index of overprocessing of the food (Sternberg et al., 1975b).

2.3 Protein Extraction using Dilute Salt Solutions

Recovery of salt soluble muscle proteins (sarcoplasmic and myofibrillar) from bone wastes is of primary importance, as the non-soluble connective tissue proteins, collagen and elastin, are of a much reduced nutritional value for humans. Sarcoplasmic proteins are readily extractable in low ionic strength (<.15) buffers or in water, whereas, the myofibrillar proteins require intermediate to high ionic strength buffers (.15 -.5) (Forrest et al., 1975). In comparison, the connective tissue proteins are only very slightly soluble in low or high ionic strength solutions. The sarcoplasmic proteins account for approximately 40% of the muscle protein and contribute to the color and flavor of the meat by reacting with the carbohydrates present in meat during the cooking (Lawrie, 1974; Hamilton, 1978). The myofibrillar proteins account for approximately 60% of the muscle protein, and are responsible for the physical properties, such as gel formation, water binding and heat coagulability.

Salt extraction of proteins from both plant and animal sources has been examined by a number of workers. Dyer et al. (1950) reported extraction of 85-95% of fish muscle protein from cod and halibut at 5°C in a neutral salt solution (pH 7 - 9) with an ionic strength of .5 - 1, which represented essentially all the sarcoplasmic and myofibrillar protein. Precipitation of the proteins occurred through a dilution with 10 volumes of water. However, Meinke et al. (1972) were unable to duplicate this, obtaining only 60 - 70% of the maximum protein extractable, even at an increased pH of 11.1.

Young (1975) extracted protein from mechanically deboned poultry meat using either neutral or slightly acidic salt solutions. Maximum extraction yields were found at a pH above 6.5 with an ionic strength above .48. The yield varied neither with temperature between 3.5 - 13°C, nor with a retention time above 2 hours. Dilution of ionic strength to .2 or lower precipitated 71 - 75% of the soluble protein. Isoelectric precipitation at pH 4.5, with an ionic strength of .4 increased the yield to over 90% of the soluble protein. Increases in the ionic strength to .3 significantly increased the percentage yield with isoelectric precipitation. However, extraction of already separated, mechanically deboned protein seems redundant. Young (1976) also extracted proteins from the bone residue of mechanically deboned chicken necks and backs, using a neutral or alkaline salt solution, producing an isolate .

containing 60 - 65% protein, 23 - 25% lipid, 5 - 10% ash, 4 - 6% moisture. The solubility and emulsifying capacity were found to increase with increased pH, and to a slightly lesser extent with ionic strength. The relatively low apparent yields would most likely be due to the high collagen content (skin, etc) in the bone residue, which is essentially non-soluble.

2.4 Hot Rendering

For many years, the only method of upgrading abattoir by-products to a useable product, was by hot rendering (Young, 1980; Wilder, 1971). However, this process which separated the fat, water and proteinaceous material, was and still is primarily used for preparation of animal feeds rather than human food. The high percentage of connective tissue, primarily collagen, which is deficient in many of the essential amino acids and totaly void of tryptophan, will considerably reduce the nutritive value of the protein product (Eastoe, 1955; Neuman and Logan, 1950; Wilson, 1969). Extensive denaturation and loss of functionality of the proteins due to the heat treatment, along with a large variability in composition and nutritive value of the product (Atkinson and Carpenter, 1970; Pritchard and Smith, 1957) reduced its appeal for human consumption. Although this processing is very severe, it has been shown that most of the functional and nutritional qualities of the rendered product is dependent on the raw materials (Skurray and

Cumming 1974; Skurray and Herbert, 1974; Bremner, 1976). Hot rendering to produce human grade proteins has been tried, though the functionality of the proteins was reduced, and the process required tighter control than usual (Anon, 1975; Loquist et al., 1962).

Young (1980) has reviewed several improvements to this basic method that have been suggested by various patents and reports. Most processes incorporate lower heat, and a shorter time with subsequent centrifugation to separate the solids and liquids, producing higher quality protein meal and fat. Hamilton (1978) mentioned a process of ultra low temperature rendering, which produces a raw meat emulsion of reduced fat content, similar in composition and perishability to MDM, though he did not specifically state the process.

2.5 Enzymatic Hydrolysis

Solubilization of meat proteins which allows for a subsequent separation of the fat, bone material can be accomplished by the use of exogenous proteolytic enzymes such as ficin, papain, bromelain, or fungal proteases (Criswell et al., 1964b; Whitaker, 1977). Along with solubilizing the myofibrillar and sarcoplasmic proteins, these enzymes also act on the connective tissue proteins, collagen and elastin (El-Gharbawi and Whitaker, 1963; Hindricks and Whitaker, 1962; Miyada and Tappel, 1956; Yatco-Manz and Whitaker, 1962). Criswell et al. (1964b) and

Connelly et al. (1966) studied the use of fungal proteolytic enzymes for protein separation and were able to produce a material with 70 - 74% protein. However, the nutritional quality of the protein was reduced due to the possible high collagen content (Miyada and Tappel, 1956, Hindricks and Whitaker, 1962). These products were suggested for use only as animal feed.

A second use of enzymatic proteolysis is the solubilization of otherwise non-soluble protein concentrates, specifically fish (Archer et al., 1973; Cheftel, 1977; Hevia et al., 1976). The initial solvent extraction methods of producing these concentrates denature the proteins, destroying many of the functional properties (Whitaker, 1977). Enzymatic proteolysis can resolubilize the proteins to enable a greater usage, though may cause the production of bitter peptides (Hevia et al., 1976).

2.6 Acid and Alkaline Hydrolysis

Acid hydrolysis solubilization of meat proteins is a more rapid procedure than enzyme hydrolysis, though it is usually more destructive. Criswell et al. (1964a) have found that a few amino acids were destroyed along with almost complete destruction of tryptophan. Isomerization of some amino acids also occurs (Provansal et al., 1975). The nutritional quality of the protein material is reduced due to the high concentration of collagen and elastin. The use of heat with acid hydrolysis further reduced the functionality of the protein. At present, this process has been only suggested for production of animal feed (Criswell et al., 1964a).

Alkaline hydrolysis (high pH, high temperature) was suggested not to be used due to slow kinetics of the reaction, possible soap formation (Criswell et al., 1964a) along with numerous undesirable chemical modifications which may occur in such severe conditions, i.e. isomerization, amino acid destruction, and new amino acid formation with possible toxic side affects (Provansal et al., 1975; de Groot and Slump, 1969).

2.7 Solvent Extraction and Separation

Solvent extraction of proteins has been used primarily in the production of protein isolates and concentrates from vegetable and fish sources. Various solvents such as isopropyl alcohol, hexane, acetone, ethylene dichloride are used alone, mixed with water, mixed together or used in succession (Anon, 1966; Criswell et al., 1964a, 1964b; Levin, 1970; Toledo, 1973; Tannenbaum et al., 1970a). The product is usually very bland with no off flavors and an almost complete loss of functionality (Whitaker, 1977), specifically solubility, which causes a gritty mouth feel. Consequently, most of the concentrates must be used in baked products, such as bread, pasta and cookies, in low concentrations (Toledo, 1973). Toledo (1973) suggested the use of a low temperature extraction to preserve some functionality, but this requires over three times the volume of isopropanol-water solution. This product has been shown to have good water and oil binding properties. Other processes, like the Viobin process (Levin, 1970), using an azeotropic mixture of ethylene dichloride, and the method of Criswell et al., (1964a) of floatation separation of fats and proteins in carbon tetrachloride, are used for the production of animal feeds. Nash and Mathews (1971) used a modified carbon tetrachloride separation method with subsequent purification by alkali-extraction, or size separation, to produce a product suitable for incorporation into baked goods, though this procedure is rather long and partially redundant. If this protein concentrate requires resolubilization, either alkali (Nash and Mathews, 1971; Tannenbaum et al., 1970a, 1970b) or enzymatic solubilization (Cheftel, 1977; Hevia, 1976; Archer et al., 1973) is required, making it a lengthy process. Possible contamination of the final product by toxic chemicals, such as carbon tetrachloride increases the risk of such a recovery method.

2.8 Summary

Each of the preceeding protein recovery methods have drawbacks which limit the feasibility of utilization. The very rapid mechanical deboning method, characterized by a minimal loss of protein functionality, is presently the most desirable method. However, it is not without weak points, such as high fat content, possible high fluoride content,presence of bone chips and loss of useable protein in the residue.

Determination of a suitable method of recovering this protein is partially dependent on the final products usage. Solvent extraction and hot rendering are both suitable methods if the product is to be used for animal feed where functionality is of reduced concern. The cost of the process is high in both cases. Enzymatic or acid/alkaline hydrolysis are both lengthy, severe processes, causing the solubilization of collagen and production of other non-desired compounds. The use of a dilute salt solution extraction can produce a suitable protein concentrate, at the expense of a very large waste water problem, caused by the protein dilution/salt concentration step.

Alkaline extraction can be conducted relatively rapidly (1/2 hour) under conditions which have a limited effect on the functional and nutritional qualities. The product is of human-consumption grade, and of high protein quality. The operational costs are low (requiring only water, NaOH, acid and raw material) though the capital equipment costs may be high. The inherent waste water production is a problem, though of limited magnitude when compared to the dilute salt solution extraction waste. The process is also relatively simple, with a limited number of steps. With this in mind, the low temperature alkaline extraction process was chosen for this work.

3. Methodology

3.1 Material Source

Mechanically deboned poultry bone residue from chicken necks, backs and spent layers processed by a Beehive mechanical deboner was obtained from a local poultry processing plant. The residue was kept at 4°C, until used (within the same day as the residue was processed). Possible detrimental effects to protein solubility caused by freezing, as found with fish products (Meinke et al., 1972) did not allow for a frozen storage of the material for subsequent usage, thus several batches had to be used. Total solids, protein, fat and ash determinations were conducted on the raw material. The non-homogeneous nature of the material required that large samples be analyzed. One kilogram samples were freeze dried, then defatted using the soxhlet procedure. The dried defatted samples were then finely ground and a protein analysis done on twelve samples. Six samples were ashed.

3.2 Alkaline Extraction

Mechanically deboned bone residue was mixed with distilled water at a ratio of 1:1.25 as suggested by Jelen et al., (1978). A 10% by weight NaOH solution was used to adjust the final pH of the mixture to pH 9, 9.5, 10, 10.5, 11, 11.5. Final pH after mixing was used as the control factor, as the pH decreased during mixing. This mixture was thoroughly mixed in a Turbula tumbler (WAB, Switzerland) for 30 minutes at room temperature (22°C ± 2°C). The mixture was subsequently centrifuged at 4000 rpm (average centrifugal force 1750 G) in a J-21 Beckman centrifuge with a JA-14 rotor, for 15 minutes at 4°C. Centrifugation occurred at 4°C to allow for solidification of the fats for easier separation. The extract was then separated from the bone residue and a vast majority of the fats, by pouring the extract through several layers of cheese cloth. To reduce possible differences caused by variances in the raw material characteristics (i.e. fat, protein, bone concentrations) alkaline extracts from four runs were mixed together and stored at 4°C prior to further processing. Total extract volume, total solids, protein, fat and ash determinations were recorded. Analyses of extracts were conducted in triplicate.

3.3 Protein Precipitation

The extract pH was adjusted to pH 4.5, 5.0, 5.5, 6.0 and 6.5 using 8.2M acetic acid, 6.1M hydrochloric acid, 7.3M phosphoric acid or 6.0M sulfuric acid. The acid was added slowly to the vigorously stirred extract in a baffled container to prevent pockets of high acid concentration. The solution was centrifuged at 5000 r.p.m. (average centrifugal force 2460 G) in a J-21 Beckman centrifuge, with a JA-14 rotor for 15 minutes at 4°C to aid solidification of any remaining fats in the supernatant. The supernatant and precipitate were separated, collected and kept at 4°C until analyzed. Triplicate analyses of the precipitates for total solids, protein, fat, water holding capacity and ash were carried out.

3.4 Water Holding Capacity

The water holding capacity of the precipitates was determined using a method described by Bouton _et al_. (1971, 1972). Three to four gram samples were centrifuged at 100,000 G (30,000 r.p.m.) for 1 hour in a type 30 rotor in a Model L2-65B Beckman Ultra Centrifuge. The expressed juice was drained and the final weight determined. Results were expressed in grams water retained per gm of dry solids. Samples were run in triplicate.

3.5 Lysinoalanine

Mechanically deboned bone residue was extracted at pH 9, 9.5, 10, 10.5, 11, 11.5 using the previously described procedure. Samples were extracted and kept for 1, 4, and 16 hours at 22°C, 35°C, and 50°C. The presence and concentration of lysinoalanine was determined at the Dept. of Biochemistry, University of Alberta (Edmonton). A Dioner (formerly Durrum) D 500 Amino Acid analyzer was used with a 50 cm x .175 cm column packed with Durrum type 6A resin (8 ± 2 u) which was 8% crosslinked. The location of LAL on the recorder was determined using a standard LAL solution.

Approximately 5 mg of fat free dry samples were hydrolyzed in 5 ml of 6 M HCl at 110°C for 22 hours in vacuum sealed glass tubes. The hydrolysate was dried over sodium hydroxide pellets in a vacuum dessicator. The dried hydrolysates were dissolved in a sodium citrate buffer at pH 2.2., and the LAL determination carried out.

3.6 Analytical Procedures

3.6.1 Total Solids

Total solids of the raw materials, extracts, precipitates and supernatants were determined using a vacuum oven method. The extract or precipitate was thoroughly mixed and 8 - 15 gm samples placed in a vacuum over at 80°C for 24 hours. Samples were run in triplicate.

3.6.2 Protein

Total nitrogen content of the raw material, extract and precipitate were conducted in triplicate using the micro-kjeldahl method (AOAC, 1975). Crude protein was calculated as % nitrogen x 6.25.

The protein content of the supernatant was determined using the Biuret method, (Gornall *et al*., 1949)

3.6.3 Fat

Crude fat content was determined in triplicate using the Soxhlet method (AOAC, 1975).

3.6.4 Ash

Ash content was determined in triplicate by incineration in a 550°C muffle furnace until obtaining constant weight (AOAC, 1975).

4. Results and Discussion

4.1 Raw Material

The raw material analysis (Table 5) shows a similar raw material composition to that used by Young (1976). Although relatively large samples of initially one kilogram were analyzed , large differences were found, especially in fat contents, between samples from the same batch, and more noticeably between batches from the same mechanical deboner. Differences in the source material used, i.e. necks and backs, necks, backs and spent layers, will affect the composition of the residue. The large amount of fat and skin associated with certain raw materials (i.e. necks) would cause an increase in the fat and collagen content. Analysis of residue from strictly necks or necks and backs was not conducted. The majority of the extractions were done on necks and backs bone residue, though the specific source was not identified for each extraction. The high protein concentration (13 - 15%) is due primarily to the collagen content from the skin and connective tissues which remain with the residue (Satterlee, 1975). It was not expected that the alkaline solvent would extract any appreciable part of this protein, as the collagen will not solubilize at the relatively low pH and temperature used (Duerr and Earle, 1974; Golan and Jelen, 1979).

Table 5

Mass Balance and Proximate Analyses of Alkali Extracted Poultry Protein, (from Mechanical Deboning Residue), at Four Processing Stages, (pH 10.5 Extraction; Basis of 1000 gms Residue; 1:1.25 Residue/Solvent Ratio)

	Mechanical Deboning Residue	Extract	Precipitate pH 4.5 - 6.0	Supernatant pH 4.5 - 6.0
Total				
wt.(gms)	1000	990	230 - 315	760 - 675
Water				
wt.(gms)	530 - 620	945 - 955	198 - 285	754 - 666
%	53 - 62	95.5 - 96.5	85.9 - 90.7	99.3 - 98.7
Total Solids				
wt.(gms)	380 - 470	35 - 45	32.5 - 29.8	5.5 - 8.2
%	38 - 47	3.5 - 4.5	14.1 - 9.5	0.7 - 1.3
Protein				
wt.(gms)	130 - 150	24 - 28	24.5 - 21.4	2.6 - 5.3
%	13 - 15	2.4 - 2.8	10.7 - 6.8	0.3 - 0.8
Fat				
wt.(gms)	160 - 200	<16	<6.4	<4.8
%	16 - 20	<1.6	<2.8	<0.7
Ash				
wt.(gms)	80 - 110	<1.5	<0.8	<0.7
%	8 - 11	<0.15	<0.4	<0.1

4.2 Extraction

Reduced processing costs of room temperature extraction along with limited benefits shown for increasing extraction temperatures above 20°C on extraction of sarcoplasmic and myofibrillar proteins, make room temperature extraction preferable. Hamilton (1978), showed no effect of increased temperature on protein yields from beef material, while Hamm and Deatherage (1960) found a detrimental effect upon increasing temperature up to 60°C. Most examples of increased yield were accompanied with a substantial increase in collagen content (Swingler and Lawrie, 1978, Duerr and Earle, 1974), along with increased risk of hydrolysis, racemization and new amino acid formations occuring. The high degree of denaturation and loss of functionality above 60°C makes high temperature extraction very detrimental. For these reasons, room temperature extraction was used.

A mixing time of 30 minutes was chosen, as work by Hamilton (1978) and Jelen et al. (1979) has shown a high percentage extraction with this time, with little improvement with increased time. Again, it also reduced possible detrimental effects such as hydrolysis.

A solvent to residue ratio of 1.25:1 was chosen to reduce waste problems connected with high supernatant (whey) production. Reducing the ratio further, was found to have a detrimental effect on the subsequent precipitation step. A high protein concentration will produce a very viscous gel when approaching pH 9, which prevents a proper dispersal of acid into the product, leading to the increased likelihood of high acid concentration pockets. Jelen et al. (1979) and Hamilton (1978) also found optimal solvent to residue ratios around the 1.25:1 range.

Results of the effect of extraction pH on the extract volume and the amount of protein extracted is shown in Figs, 3 and 4. Fig. 3 shows the results from two samples from the same batch of raw material, while Fig. 4 shows the results from three samples from two different batches. Both curves exhibit a similar trend with a maximum extract volume between pH 9.5 and pH 10. The drop in extraction volume below pH 9.5 is attributed to the substantial increase in viscosity of the protein slurry which was similar to that found by Jelen et al., (1978). Hamilton, (1978) presented a curve showing no decrease in extract volume above pH 10, though he choose to ignore a point on his graph, showing some peaking near pH 10. The difference in source material (beef or poultry) may also account for this difference. Meinke et al, (1976) showed a similar trend using fish as a source material, though the analysis appeared to be conducted over larger pH intervals, thereby possibly missing the slight rise. Increasing the pH above pH 10 may cause excessive swelling in the connective tissues, which could account for an increased entrapment of the extract, thereby reducing the extract volume. This would be more noticeable in the mechanically deboned residue than in the ground meat samples used by Hamilton and Jelen due to the higher content

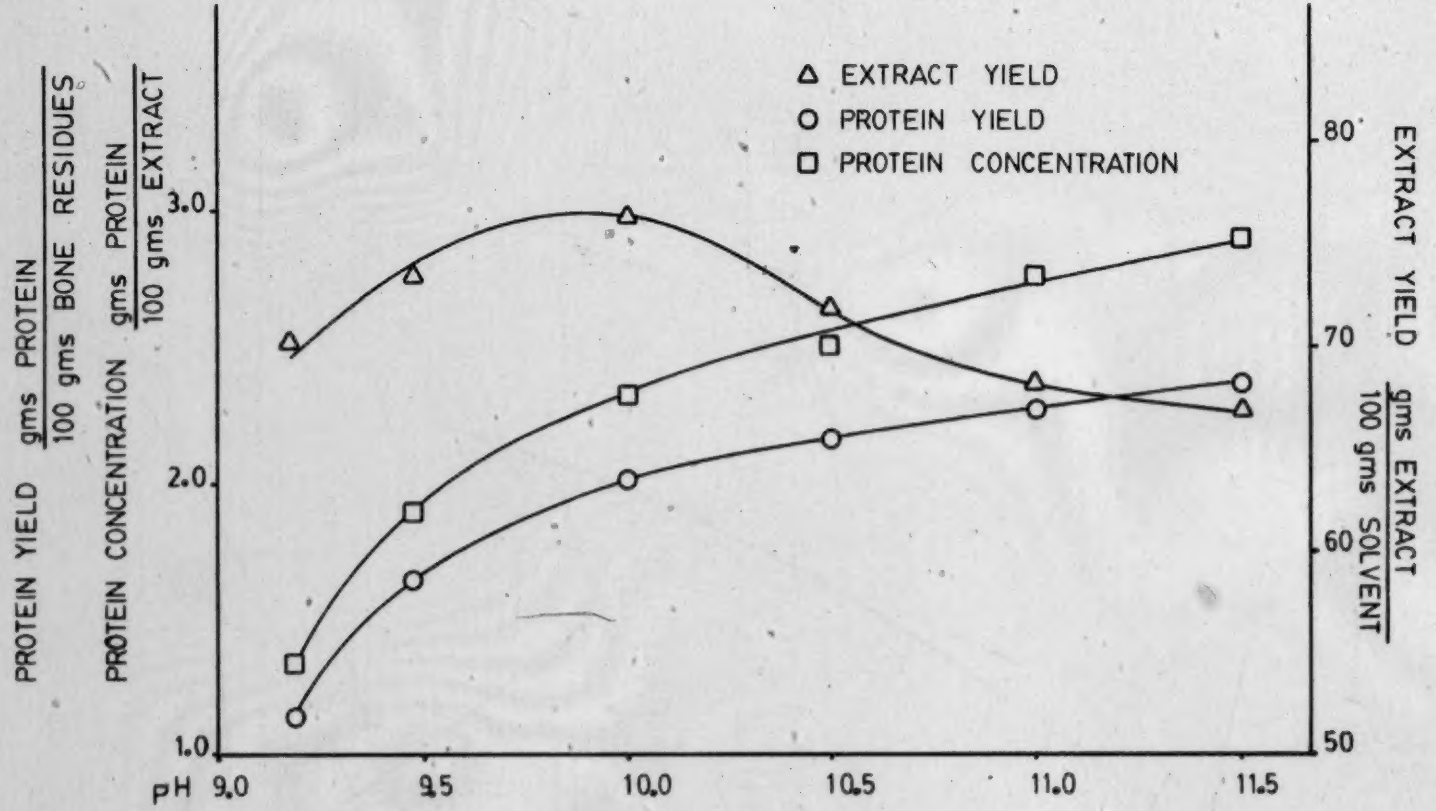

Figure 3 Effect of pH on Extraction Yield, Protein Yield, and Protein Concentration, Batch 1.

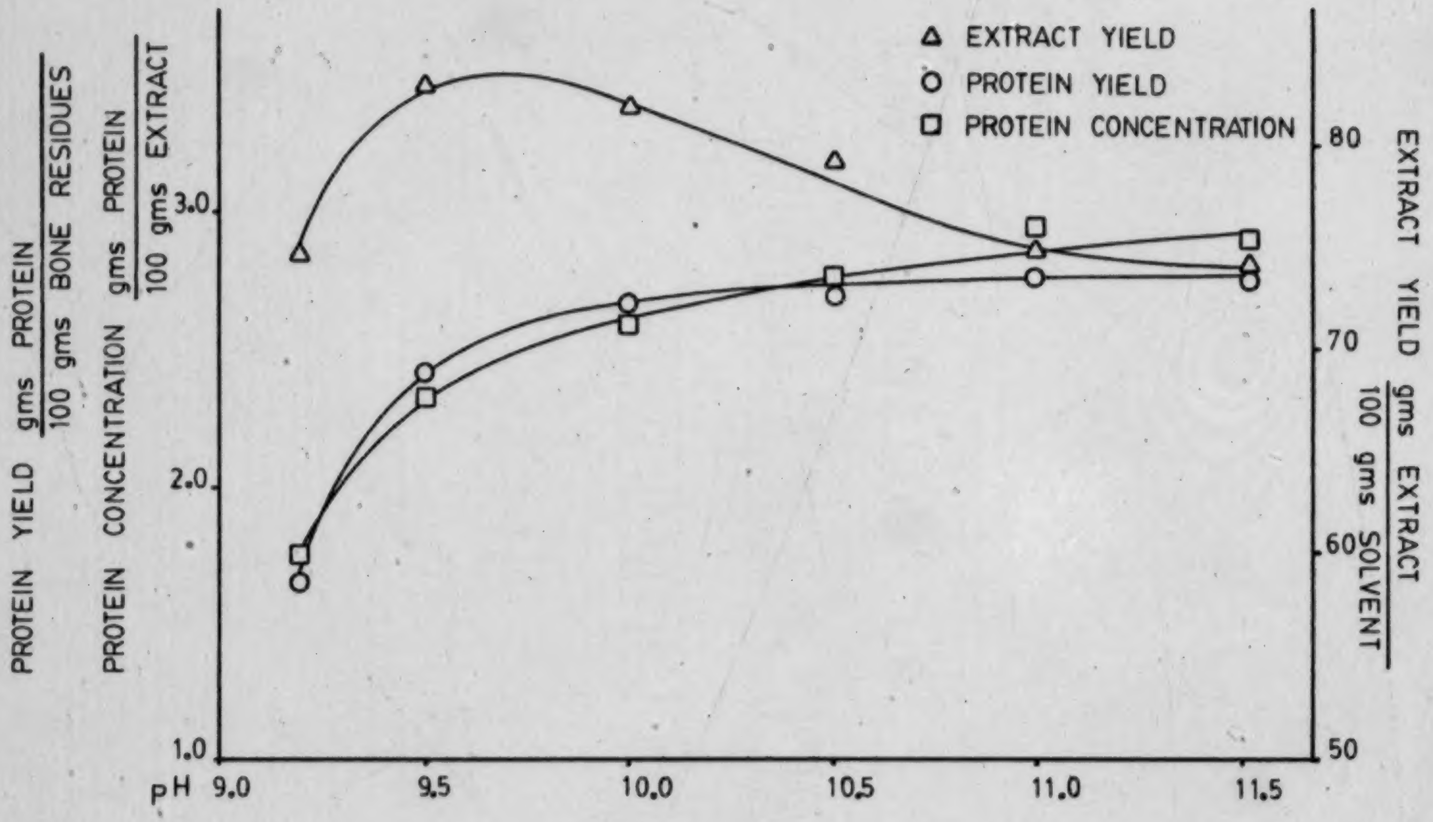

Figure 4 Effect of pH on Extraction Yield, Protein Yield and Protein Concentration, Batches 2 & 3.

of skin.

The relative protein yield (grams of protein/100 grams of bone residue) was dependent on the extract volume and the concentration of the protein in the extract. Increasing the extract pH above pH 10.5 to pH 11 or 11.5 showed little (Fig. 3) or no (Fig. 4) increase in the total protein extracted, due to the decrease in extract volume. Extracts above pH 11.5 were not obtained. The increased probability of hydrolysis, racemization or new amino acid formation occurring, connected with these elevated pHs makes this pH range unattractive. Subsequently, an extraction pH of 10.5 was chosen for use in all the remaining work.

The difference in the two curves is attributed to differences in the source material and their compositions. Increased bone, skin and fat content (causing a subsequent drop in extractable protein) may cause an increase entrapment of extract in the centrifuged bone and skin residue. The lower protein content in the extract, correlating with the reduced extract volume shown in Fig.3 agrees with this.

During the extraction mixing process, it was also noted that a correlation existed between the state of the fat globules and the pH. Below pH 11.0, the fat remained in small globules, interspersed throughout the media. However, at a pH of 11 or above, the fat globules agglomerated together, forming globules with a diameter of 1 to 2 cm which rose to the surface if the mix were allowed to sit for several minutes before centrifuging.

4.3 Precipitation

A high protein concentration coagulum (precipitate) and a low protein concentration supernatant ("whey") were produced upon reducing the pH of the extract. Fig. 5 and Table 6 show the effect of varying the final pH between 4.5 and 6, and using different acids (acetic, hydrochloric, phosphoric and sulfuric acid) on the total solids of the precipitate and the % protein (dry weight). The % total solids follows a general protein solubility curve with the lowest solubility between pH 4.5 and 5, similar to that for fish protein (Meinke _et al_., 1972), and beef protein (Jelen _et al_., 1979; Hamilton, 1978). The water holding capacity of the protein coagulum followed a curve (Fig. 6) similar to that found by Hamm (1960) (showing bound water vs pH of beef muscle), with a minimum water holding capacity near pH 5.

Differences between acid types on total solids of the precipitate were minimal, though phosphoric acid appeared to produce a higher total solids at pH 5 and pH 5.5 (significantly different at 5% level) than the other acids. Acetic acid also showed a lower total solids at pH 4.5 (significantly different at 5% level).

The percentage of protein in the dry matter does not appear to be dependent on the precipitation pH (Table 6). However, hydrochloric acid does show a higher percentage of protein at pH 4.5, 5.5 and 6.0, (significantly different at

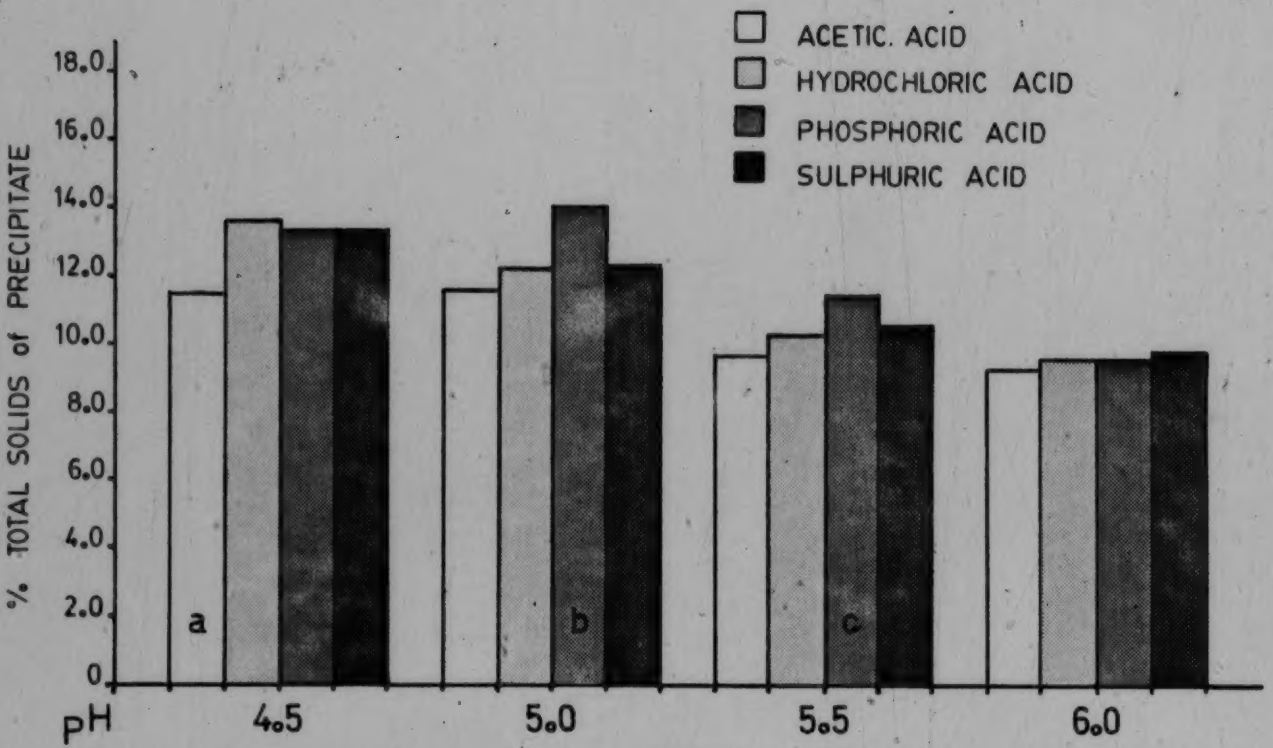

Figure 5 Effect of Precipitation pH and Acid on % Total Solids of Precipitate. a,b,c, significant difference ($p \leqslant 0.05$) between acids at same pH.

Table 6

Effect of Precipitation Acid on the Protein Content of the Precipitate (d.w.)

	pH			
Acid	4.5	5.0	5.5	6.0
Acetic	71.3 (.8*)	74.1 (.8)	73.5 (.6)	73.0 (.7)
Hydrochloric	75.2[a](1.1)	75.4 (.3)	75.4[b](.6)	75.3[c](.6)
Phosphoric	71.6 (.5)	73.4 (.9)	72.9 (.3)	72.7 (.8)
Sulfuric	72.8 (.5)	74.2 (.6)	73.5 (.4)	73.2 (.8)

* standard deviation

a,b,c significantly different at 5% level within each column

analyses were run in quadruplicate

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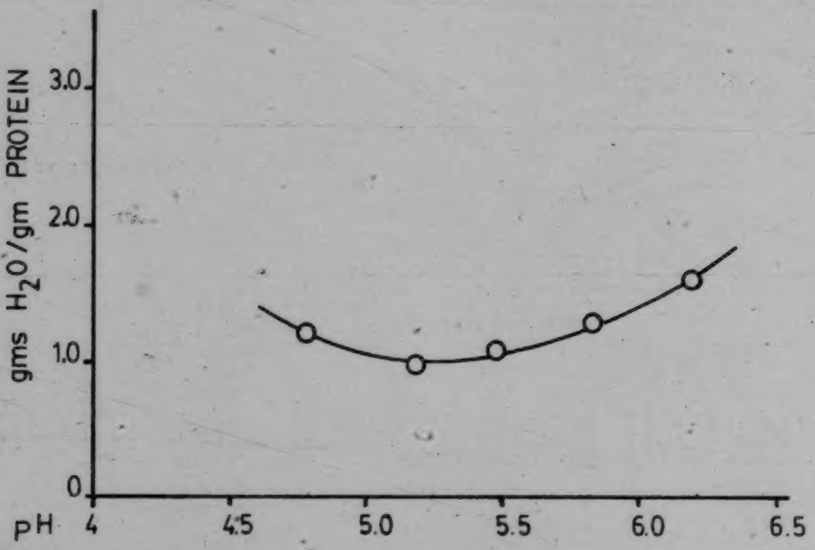

Figure 6 Water Holding Capacity vs pH.

5% level) than the other three acids. Jelen et al. (1979) produced a coagulum with a higher protein content (approximately 80%). However, this was recovering protein from beef with a higher initial protein concentration, of which there would be less collagen. The raw material (M.D.P.R.) used in this research would also have a higher bone content.

Figs. 7 and 8 show the effect of extraction pH, precipitation pH, and acid type on the protein concentration of the supernatant and the volume of the supernatant. The specific points shown are for a hydrochloric acid precipitation, though the 95% confidence limits are shown combining the results of all four acids. The hydrochloric acid supernatant volume and protein concentration appear to deviate only slightly from the 95% confidence limits at the higher pH (pH 6.3 - pH 6.7).

The supernatant exhibits close to a 250% increase in protein concentration between pH 5 and pH 6.6, whereas the volume decreases by less than 20%. The total protein in the supernatant at pH 6.6 is therefore approximately twice that at pH 5.0. As seen in Table 5, this can account for 10% to 20% of the total extracted protein, which would be a significant loss. Consequently, the total precipitated protein decreases at the higher pH. A change in pH from pH 5.0 to pH 6.6 would effect a 12% decrease of precipitable protein.

The protein lost to the supernatant is of significance

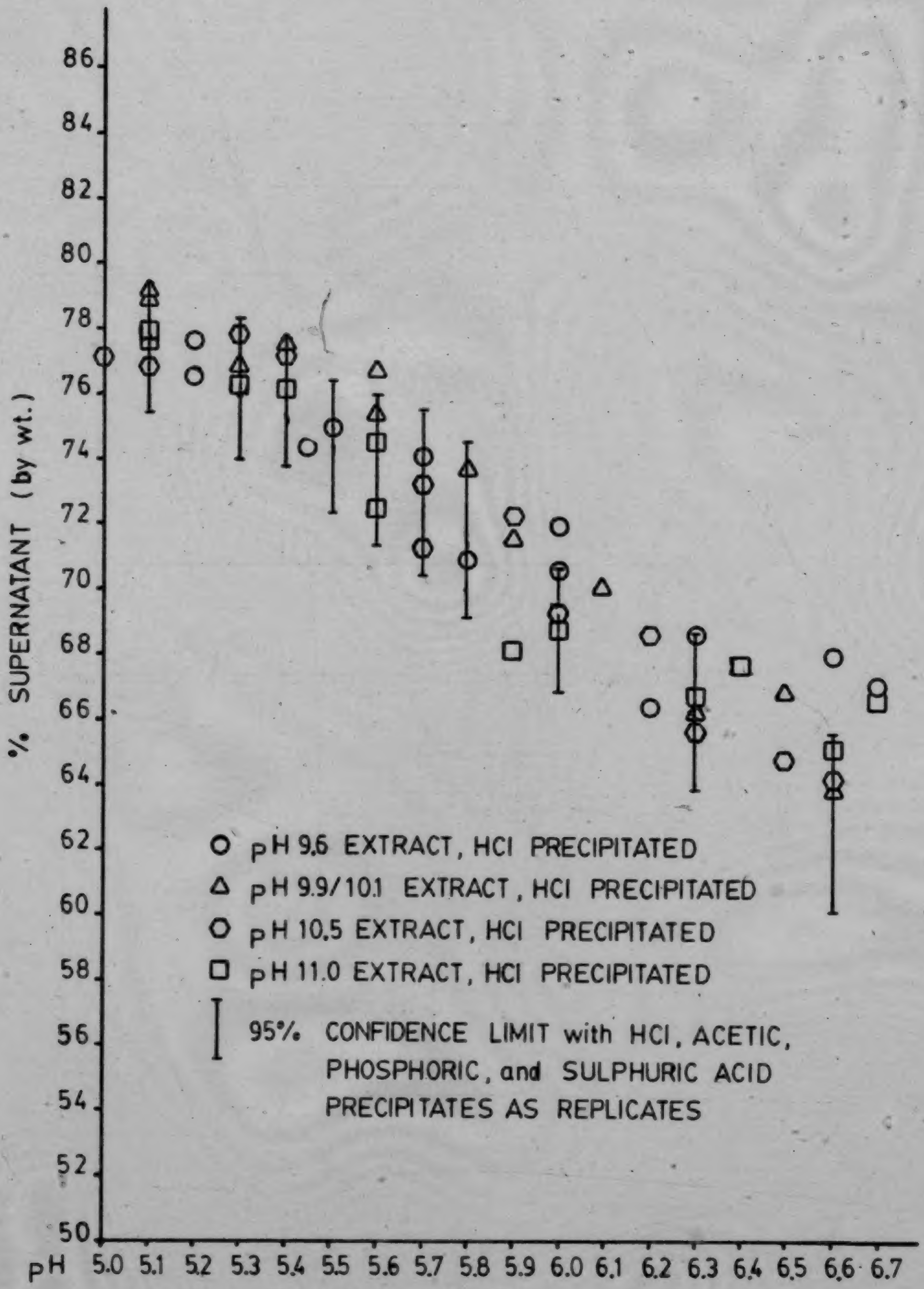

Figure 7 Effect of Extraction pH, Precipitation pH and Acid on Supernatant (% of Total Weight).

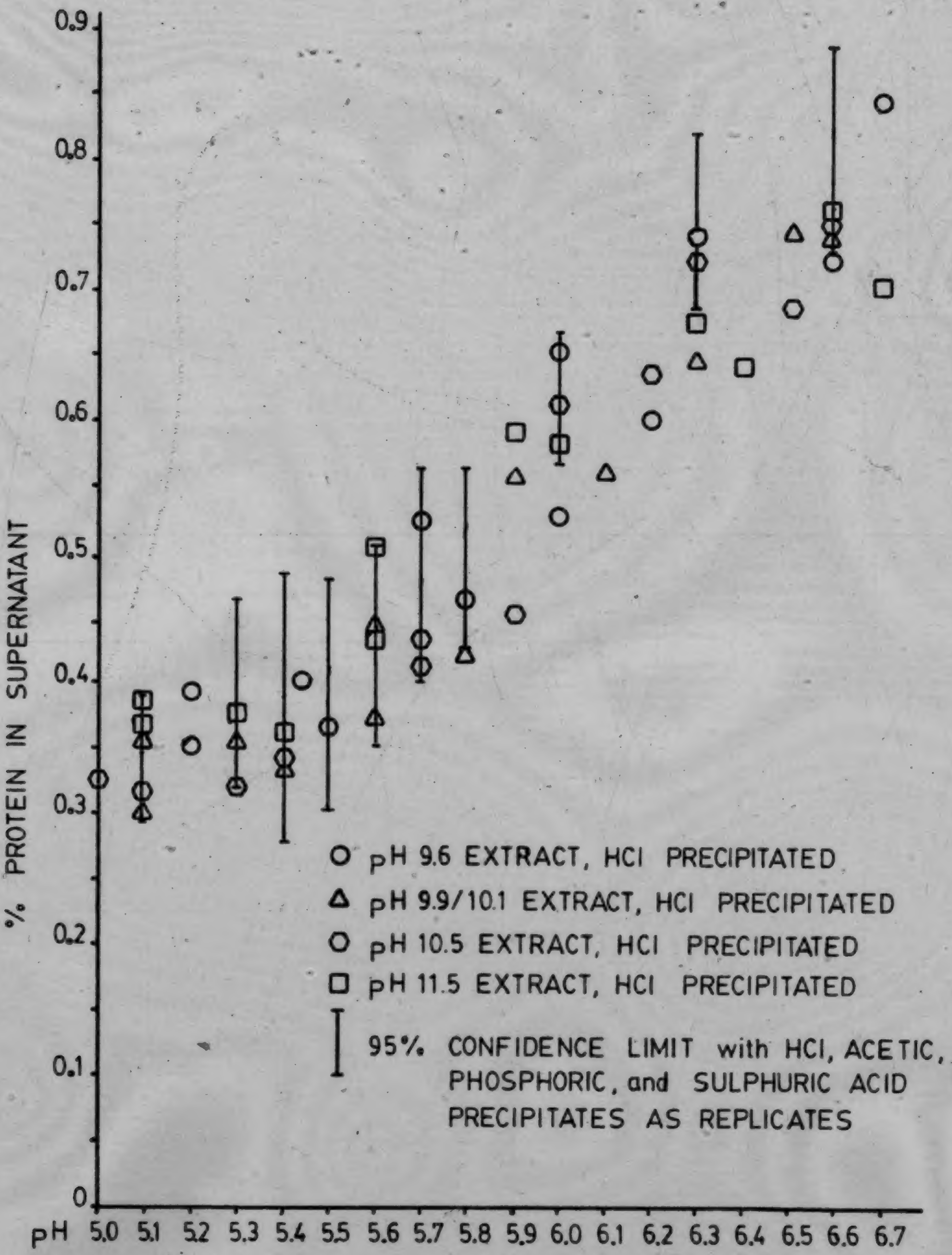

Figure 8 Effect of Extraction pH, Precipitation pH and Acid on % Protein in Supernatant.

for two major reasons. Firstly, the loss of high grade human food protein, reduces the efficiency of the process. The second concern deals with the utilization or treatment of the supernatant "whey". The high protein concentration will increase the cost and difficulty of waste water treatment. This has to be kept in mind when determining which precipitation pH to use, or whether the process is economically feasible.

There are a few possible methods of utilizing or reducing the problem of the supernatant, though these were only very briefly explored. The majority of the non-precipitable proteins are the sarcoplasmic proteins (Hamilton, 1978), though there may be some myofibrillar proteins present due to their change in structure by alkaline treatment. The increased salt concentration (NaOH + HCl) would also aid in the solubilization of some of the salt soluble proteins. The proteins would therefore have noticeably different functional characteristics than the precipitated proteins, and may not be usable under similar conditions.

Heating the supernatant to above 65°C caused a thermally induced precipitation of the majority of the proteins. However, this is a very energy intensive method, and greatly reduced the protein functionality. Recycling of the supernatant as a solvent may also be feasible, though this may be limited by microbiological considerations or concern over the numerous alkaline treatments and their effect on the protein. An increase in salt concentration would also occur in the supernatant, which would cause an increased solubilization of the salt soluble proteins.

The ash and fat concentrations at the four stages of the processes are listed in Table 5, along with the total weights of each component, assuming a basis of 1000 grams of raw material and 1250 grams of water. A very limited amount of the calcium from the bone was present in the extract after the initial centrifugation of the extract. The fat concentration was dependent on how carefully the extract was removed from the centrifuge bottles, and not on acid type, extraction pH or precipitation pH. The secondary centrifugation of the precipitate from the supernatant at low temperatures (4°C) allowed for a further separation of some of the remaining fat.

The addition of acid to the precipitate had to be controlled carefully to prevent the formation of high acid pockets causing extreme denaturation, and to prevent overshooting of the desired pH. Plate 16, shown on page 151, shows small protein blisters, caused by acid pockets, in a piece of texturized protein. This pocketing can have detrimental effects on the subsequent texturization process. Overshooting the desired pH will require the addition of NaOH to correct the pH, which in turn will increase the ionic strength of the solution, causing an increased concentration of the salt soluble proteins in the supernatant.

4.4 Formation of Lysinoalanine

Under the alkaline extraction conditions (pH 10.5, 22°C, 30 minutes mixing) used in this research, the formation of lysinoalanine (LAL) was found to be non-discernable, or well below the detectibility limit of 75 ppm (Fig. 9). The formation of this new amino acid was shown to be dependent on the retention time in the alkaline state, the alkalinity of the extract and the temperature of the extract. Low alkaline extracts (pH 9.2 and pH 10.0) show the formation of a discernable amount of LAL only at 50°C with a retention time of 16 hours. Such a situation is unlikely to occur with this process, as the extract produced a very obnoxious odor when held for 16 hours at all temperatures. The odor is also present after 4 hours at temperatures greater than 35°C, and does not disappear upon acid precipitation.

Extraction at pH 10.7 showed an increase of LAL formation at all temperatures, though this was only discernable after 16 hours retention at 22°C, 35°C and 50°C and 4 hours retention at 50°C. A four fold increase was seen at 50°C, pH 10.7 over 50°C at pH 10.0. Increasing the extraction pH to 11.5 has a very severe effect on LAL formation, particularly at 35°C and 50°C, at both 4 and 16 hour retention times. Again, a four fold increase in LAL concentration was found at 35°C and 50°C for 16 hours and at 50°C for 4 hours.

The general trend of lysinoalanine formation found in

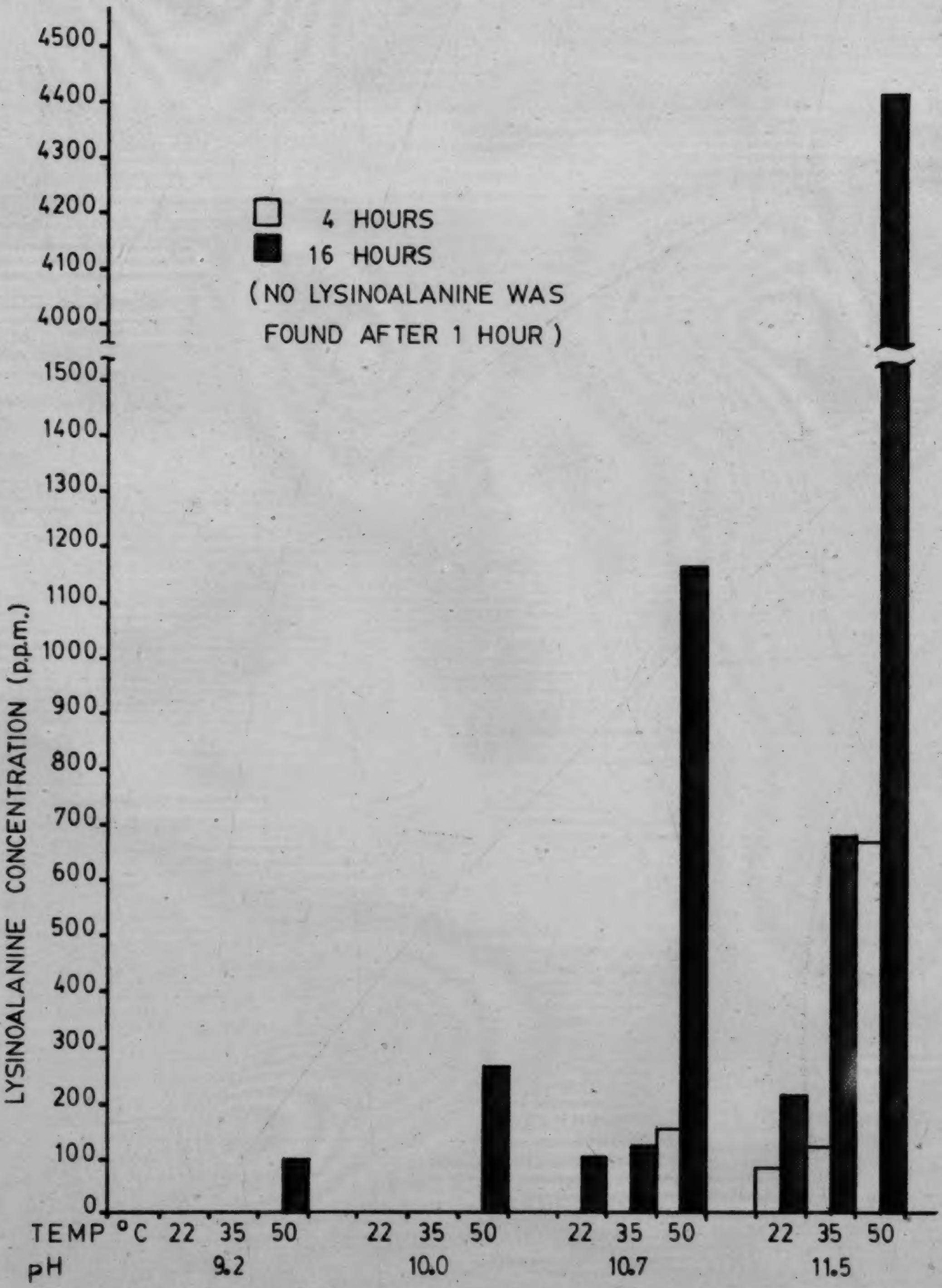

Figure 9 Effect of Extraction pH, Retention Time and Temperature on Lysinoalanine Concentration.

these conditions follows those shown by Swingler and Lawrie, (1978), Sternberg et al., (1975), and Slump, (1978). The more severe the alkaline extraction conditions, the more likely the formation of LAL and subsequently, the possible formation of other new amino acids such as lanthionine and ornithinoalanine (Aymard et al., 1978). It may also be a measure of the probability of modification to other amino acids, i.e. racemization. By using conditions unfavorable to the formation of LAL, one is also reducing the likelihood of other undesirable reactions occurring.

The optimal extraction conditions for the extraction of proteins from mechanically deboned poultry, as determined in this research, are outside of the range of conditions found to form LAL. A deviation from these conditions ie. $10.0 < pH < 11.0$, $15°C < temperature < 30°C$, 30 minutes $< time < 2$ hours, would still not be likely to form a discernable concentration of LAL, as shown from Fig.9 . This would suggest that this process does not have a detrimental effect on the amino acids of the extracted proteins.

4.5 Remarks

Specific choice of optimal conditions will be dependent on the raw material and intended utilization of the final product. As will be shown in the next section, the nature of the protein product will greatly affect further processing. Although maximum recovery of extractable proteins is desired, combined with a low protein supernatant waste material problem, any subsequent processing must also be taken into considerations.

Under the extraction conditions utilized in this research (pH 10.5, 30 minutes, 22°C) the formation of lysinoalinine was not apparent. Even with considerable alterations to these conditions, lysinoalanine was not found, suggesting that the processing environment was not very severe.

Choice of suitable acids was shown to be dependent on availability or cost, as the four acids, (hydrochloric, acetic, phosphoric, and sulfuric) produced similar results. The only major difference existed in the method of acid addition as both acetic and phosphoric acid did not require dilution, while the hydrochloric and sulfuric acids were diluted 1:3.

The feasibility of this method of protein recovery from this raw material is entirely dependent upon economic considerations. Industrial scale equipment is available for the mixing and separation stages, and the product is very suitable for human consumption. As the protein source is essentially free (the residue may still be sent to the rendering plant) the cost considerations involve

1) cost of NaOH, acids, water
2) equipment and operating costs
3) cost of waste water treatment.

As this process is relatively non-energy intensive, the processing costs and the product should become more attractive in the future.

The product should have a high emulsification capacity and would therefore be an asset to comminuted products, and could subsequently replace high cost protein materials. As an animal protein, the product contains the essential amino acids which may be absent in vegetable proteins, and is likely to have a better public acceptance than vegetable proteins. Due to the low fat content and the high protein concentration, this protein source is also suitable for further modifications, such as texturization processing as discussed in following section.

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Part II

Freezing Induced Texturization of Protein

5. Part 2: Freeze Induced Texturization of Protein

5.1 Introduction

Optimal utilization of protein resources for human consumption will become of greater concern as the cost of food production, particularly for protein rich foods, increases with respect to income. As North Americans will approach world prices in certain preferred protein sources (red meats, poultry, etc.), the cost advantages of other protein sources will become more pronounced. Unfortunately, in their original state, many of these proteins are unacceptable for human consumption, thus requiring some physical and/or chemical modifications. The nutritional value, functionality and state of the resultant material, along with consumer preferences will dictate whether the material can be used supplementary to existing protein foods, as an analog or simulation of traditional protein foods, or as a novel protein product.

Although the nutritional values (i.e. quantity of proteins, biological values and availability) are of primary concern in new protein products, the acceptability of the product is more dependent on its functional properties, in particular texture, flavor and color. Products of high nutritional value are of little importance if there is no consumer acceptance.

The study of functional properties of food proteins covers a wide spectrum of physicochemical properties which are affected by the processing or state and relationships of the proteins in a food, usually determined in the final product.

The second and major part of this work deals primarily with one property of a chicken protein concentrate: the ability to form a modified texture by fibre formation, through freezing and its inherent ice crystal formation.

5.2 Research Objectives

Modification of the textural structure of proteinaceous materials to simulate the fibrous nature of muscle tissue has been practised industrially using only two methods: spun fibre and extrusion. Both methods have certain advantages and disadvantages in cost, product quality, product stability, product range, technical knowledge requirements, etc.. Freeze-fibre formation is an alternative to these processes, though it has not been investigated beyond patent formulations, and therefore its characteristics, advantages and drawbacks are only vaguely known.

The objective of the second part of this work was to use the protein precipitate slurry studied in Part 1 as the material source for the freeze fibre formation process. The effects of the following parameters on the fibre formation were investigated:

a. Freezing rates.

b. Design of the freezing mould; Semi-infinite cylinder versus semi -infinite slab.

c. Protein precipitate pH.
d. Type of acid used in precipitation.
e. Total solids prior to freezing.
f. Heat-setting of frozen protein matrix.

The effects of these parameters on the freeze-texturized product were analyzed by:

a. Textural analysis using a Kramer Shear Cell in an Instron Universal Testing Instrument.
b. Total solids analysis of the product prior to freezing and after freezing and heat setting.
c. Fibre density analysis with the use of macrophotography.
d. Retort stability of the product.

6. Literature Review

6.1 Protein Texturization

Protein texturization is generally understood to mean the modification of the physical and/or chemical structure of a protein-rich material to simulate in appearance, structure, shape, mouthful etc. a more conventional food, ideally meat. The modified material must also be able to retain its structural entity or texture after cooking or other processes usual to food preparation. According to the U.S.D.A. (1971), "textured vegetable protein products are food products made from edible protein sources and are characterized by having structural integrity and identifiable texture such that each unit will withstand hydration in cooking and other procedures used in preparing the food for consumption".

In most descriptions of texturized proteins, the structure of the product is usually compared to the regular arrangement of muscle fibres in meat. Consumer acceptance of such restructured proteins appears to be heavily dependent on the simulative effect of the process and material on fibre formation. Pyke (1970) covers the general sentiments in the following statement; "In order for synthetic or isolated proteins to compete with and complement meat, they will need to be fabricated into something not entirely dissimilar to meat". Perhaps, if the major objective was to introduce a novel protein product of high functional and nutritional qualities, which incorporates only some of or most of those found in meat, rather than strictly attempting to simulate meat, a greater diversity of textured protein products could be developed.

The multivariably affected functional properties of proteins combined with the effects of different textural modification processes affect the ability to simulate the aligned fibrous nature of meat, along with some of its functional properties. This dictates the textural and economic success.

The original state and functional properties of a protein source dictate the extent to which subsequent modifications can texturally alter the material. Chemical modifications of the protein structure, particularly dealing with covalent bonds, to allow for easier and greater subsequent manipulation, are limited due to the immensity of the ensuing required safety analyses to satisfy regulatory standards. Therefore, most alterations are limited to some secondary, though predominantly tertiary and quaternary structures. Thiol-disulfide interchange reactions are the only changes in covalent structures suggested to occur in the textural modification process of fibre spinning, extrusion (Kinsella, 1978; Huang & Rha, 1975) and freeze-fibre formation (Hashizume et al., 1971, 1974a, 1974b).

6.2 History of Texturized Proteins

Textural modifications to proteinaceous material has been practised for centuries as a method of preservation and to increase the palatability of certain foods. Gluten in bread and coagulated casein in cheese are two common examples. Traditional oriental foods such as tofu, kori-tofu, sufu, tempeh, etc., are all texturally modified products made from soybean protein. Of particular interest to this work is the production of kori-tofu, (originating in 16th century Japan), by freeze modifying tofu, a soft gelatinous bean curd. Intended as a means of preservation, this process also considerably alters the textural structure of the material.

Attempts to produce a meat-like substitute originated in the late 1800's due to the work of Kellogg, a Seventh Day Adventist who was attempting to simulate some of the meat texture and flavor in a vegetarian diet (Kinsella, 1978). His 1907 patent stated: "the main objective of this work is to provide an improved food product which is very palatable and nourishing and one adapted for use as a meat substitute", and which, "can be economically and satisfactorily produced". (Kellogg, 1907). This, in effect, describes the objective of all meat simulation processes. Utilizing casein, gluten, and vegetable oil, the mixture was placed in cans and cooked as a comminuted product. The product subsequently has a "meaty consistency and is quite like some meats in flavor". Other meat substitutes simulating comminuted products have since been introduced and patented using similar procedures (Wrenshall, 1951; Circle & Frank, 1959).

Subsequent work on textural modifications of proteinaceous material has dealt almost in entirety on non-meat protein sources, such as casein, gluten, etc., and recently primarily soy protein. Alteration of initially non-palatable meat protein sources has only been investigated recently (Kinsella, 1976; Clark, 1978), mechanical deboning being considered as a means of protein recovery and not textural modification .

Fabrication of protein fibres was introduced by Boyer (1940) based upon the knowledge and technology of the textile fibre-spinning industry. The first industrially practical patents on this system were not awarded until 1954 and 1956 (Boyer, 1954, 1956). Gutcho (1977) has compiled the numerous unique methods and modifications to existing methods which have subsequently been introduced and patented, to improve in some manner, the existing texture of a proteinaceous material. Of these, only two, spun fibre and extrusion, have gained industrial acceptance, partially due to the experience and knowledge obtainable from existing analogous textile fibre-spinning operations and thermoplastics and other food (starch) extrusion processes.

6.3 Fibre-Spinning

Based upon textile fibre-spinning technology, Boyer (1940, 1954, 1956) initiated research and produced the first patents for a spun-fibre method of producing protein fibres, or more precisely filaments, which could subsequently be bound together to simulate the muscle structure of meat. Present industrial processes are predicated upon Boyer's work, with some modifications dealing primarily with the protein source, dope preparation, coagulating bath and subsequent treatment of fibres (Gutcho, 1977).

Boyer's process of wet fibre spinning involves solubilizing edible protein in an alkaline solution with isoelectric point precipitation in a filamentous form, in an acid saline coagulating bath. The fibres are then bound together in bundles with various binders and fats to simulate muscle. The basic process has been discussed in detail by Boyer, (1956), Hartman, (1978), and Kinsella,(1978).

A number of different protein isolates (95% to 98% protein) have been investigated, individually or in combination, including soybean, rapeseed, sunflower seed, cassin, whey, and lung and stomach tissue from beef (Kinsella, 1978; Hartman, 1978; Young and Lawrie, 1974). The product characteristics were found to be dependent on the protein source. The basic structure requirements of polymers for fibre formation are, an average molecular weight between 10,000 and 50,000, a high degree of linear symmetry, long chain lengths, absence of bulky side groups, and a high content of evenly spaced polar groups (Hartman, 1978).

The protein isolate is then used to make a 15% to 20% protein solution, pH 10 to 12, with the most critical factor, viscosity, being between 30 and 350 poise, (Kinsella, 1978). The elevated pH can be detrimental as it makes the protein susceptible to hydrolysis, racemization or to the formation of new amino acids. Some coloring, flavoring and other functionality modifying agents may be added at this point. However, the inclusion of non-miscible compounds, like lipids, can cause weak points in the fibres. Fibres with greater than 30% additive materials show a significant reduction in tensile strength (Boyer, 1956).

The dope is pumped under pressure through a multi-orifice spinneret head (0.0075 to 0.075 mm diameter orifices with 1,000 to 15,000 orifices per head) into an acidic (pH 2 to 3) bath containing 10% salt. Upon exitting the die, the fibre undergoes a swelling phenomena. The fibres are collected on reels after passing through several baths containing neutralizing solutions, lipids and binding and flavoring agents. The fibres are kept under tension, causing an increase in protein alignment, fibre length and tensile strength. Unstretched fibres remain kinky, weak and inelastic (Kinsella, 1978).

These fibres are collected in bundles after being coated with binders such as albuminoid proteins, gelatin, gums or processed starches, and are heat set, causing the fibres to bind together by gelation, adhesion, or chemical crosslinking. Although it has been shown that, under appropriate temperatures and pressures, certain fibres require no binders, the majority of fibres require some form of binding agents.

The majority of spun fibres are used as meat analogs, which can withstand slicing, grinding, freezing and drying. The dry product is relatively stable against microbial contamination in storage, though prolonged storage can cause greying, toughening, and loss of resilience and elasticity. Various mechanisms for the biosynthesis of protein fibrils have been reviewed by Huang and Rha (1974), Lundgren (1949), Wormell (1954), Hearle and Peters, (1963). Globular proteins are initially unfolded to random coils by alkaline denaturation. In the subsequent spinning process as the dope is extruded into the acid bath, pressure and shearing forces cause alignment of proteins along the long axis with subsequent cross-linking and bonding. Stretching of the fibre after extrusion is believed to produce a more uniform, organized alignment of the proteins in the direction of the fibre (Lundgren, 1949; Huang and Rha, 1974).

At present, there are a few operating spun-fibre plants (Miles, General Foods, General Mills) which have shown that the process is industrially viable. However, the process has a number of inherent difficulties and disadvantages.

Fibre spinning is a complex technology, requiring highly trained technicians and sophisticated machinery, which in turn makes the process costly and limits the market. The quality and characteristics of the protein isolate have been found to vary between batches, requiring modifications to each run (Kinsella, 1978). Insoluble particles can cause breakage in fibres, or clogging of spinnerets, thereby enforcing the use of high quality proteins. Intensive alkaline treatment can also be detrimental to the proteins. A major drawback to public acceptance is the lack of an entirely bland flavored protein source.

6.4 Extrusion

Textural modification of edible proteins by extrusion gained prominence as a viable, less expensive, and less sophisticated alternative to the already semi-established spun-fibre process. Initially, the extrusion-cooking process was applied primarily to expel oil from oilseeds, gelatinize starches and mix additives (Kinsella, 1978). Extrusion processing of proteins to simulate meat-like products originated with work by Anson and Pader (1957,1958, 1959), who produced a filamentous "chewy gel" protein material. Until the introduction of a patent by Atkinson (1970), subsequent methods of protein extrusion produced a puffed product with air vacuoles which were non-oriented; thus the material may have had some of the mouthfeel of meat, but lacked the fibrous structure. Atkinson's patent (1970) altered this by introducing a method by which longitudinal vacuoles were produced, making a product with an oriented fibrous structure, or by his terms, a "plexilamellar structure". Presently over 60% of the protein texturizing capacity in the United States is licensed under this patent (Kinsella, 1978). Furthermore, the general extrusion process accounts for over 95% of the texturized plant proteins.

Protein texturization by extrusion involves applying a shearing force at elevated temperatures to a moist proteinaceous material, causing gelatinization and subsequent formation of a plasticized mass, caused by protein polymerization. This material is then forced under high pressure through a limiting orifice, or die, into an area of reduced pressure and temperature, causing an expansion or swelling of the material if the pressure gradient is large, plus a thermal setting of the material. The basic process and major controlling factors are described below. Of all the factors involved, the most important determinant of the final product and its properties is the feed material and its moisture content, (Clark, 1978).

Although the purity of the protein concentrate (40% to 70%) is not as critical as with the spun fibre process, it has been shown that different proteins and concentrates will affect the texture. Highly denatured proteins have been shown to produce a non-cohesive product. At present, soy flour, soy grits and soy protein concentrates are the primary sources although peanut, cottonseed, wheat gluten, triticale, yeast protein, distillers grain and meat and meat by-products have been investigated alone or in combination with each other (Kinsella, 1978; Clark, 1978). Use of full fat vegetable flours have been shown to decrease the degree of protein denaturation, though the presence of lipids tends to weaken the exudate. Industrial utilization of meat and meat by-products has been limited strictly to the pet food industry (Clark, 1978), although patents have been awarded for the extrusion of secondary protein sources such as meat by-products (Baker et al., 1975).

The moisture content has been found to be the most critical parameter affecting the structure, with a 15% to 40% content preferred. A low water content can produce a dense, non-expanded product, while too high a water content can produce a weak product, subject to collapse and hardening (Conway, 1971a,b). The water content also affects the gelatinization, cohesion and vapor release of the product, along with the mechanics of the extruder.

Acid (pH 5-6.5) products have been shown to be dense, chewy, harden slowly and exhibit poor rehydration, whereas alkaline pHs are soft, less chewy, less plastic, looser and more hydratable (Kinsella, 1978).

Inside the extruder, the proteinaceous material is forced along by a screw, while being heated by frictional heat, heating jackets or screws, or direct steam injection. The minimum critical temperature required is 120°C (Kinsella, 1978). Increasing the temperature to 150°C increased rehydratability, fibre formation and orientation, and tensile strength (Cummings et al., 1972). There is conflicting data as to the effects of temperatures greater than 150°C (Cumming et al., 1972; De Man, 1976; Maurice et al., 1976). The cooking process may sterilize or pasteurize the product, inactivate detrimental enzymes and cause some volatilization of off-flavors and off-odors (Kinsella, 1978).

A pressure increase is produced by a positive displacement pump mechanism or a decrease in the space between the screw tape and the barrel. Preferred pressure is between 2700 and 4100 KPa.(Kinsella, 1978).

Motion within the barrel can best be described by unwinding the screw, which would form a long narrow channel with a flat plate moving at an angle across it. Therefore, the particles follow a helical pattern, causing good material uniformity with good heat transfer. The addition of energy and shear force will cause an increase in the apparent viscosity of the dough as it moves down the barrel, which is attributed to a network formation amongst different food components. Subsequently, the proteins become aligned in sheaths in the direction of flow. However, if the shear becomes too severe or extensive, it may cause a breakdown of the structure, reducing the final cohesiveness (Clark, 1978).

Upon exiting the extruder, the material undergoes a rapid pressure and temperature drop as water vaporizes very rapidly. The orientation of these steam created air vacuoles is dependent on the nature of the die. Use of extended length dies (Atkinson, 1970), causes the formation of long, axially oriented vacuoles, producing a parallel oriented fibrous product.

The dried product has an extended shelf life of over a year, has a low bulk density, low storage and handling costs and can be infused with fat, coloring and flavoring agents, and vitamins upon rehydration.

In comparison to spun-fibres, several cost analyses have shown this to be a more advantageous process, though a poorer fibrous product is obtained (Horan, 1974,1977). This process is also energy intensive, which will cause obvious cost increases. The product is intended for use primarily as a meat extender, though recent techniques have allowed utilization as meat analogs. Concentrations of greater than 10% extruded protein in meat products have been found to detrimentally effect the appearance and flavor of meat patties (Kinsella, 1978). Perhaps the major drawback is public acceptance of meat analogs and extenders, especially those which differ noticeably from meat (Robinson, 1972).

6.5 Alternate Processes

Gutcho, (1977) lists a number of patents which relate to other methods of protein texturization. Kinsella (1976; 1978) and Horan (1974) discuss some of these processes. Most of the new methods are modified extrusion or spun-fibre processes, or some combination of the two, attempting in some manner to improve the product or to simplify the process. A few improvements are worthy of note. The addition of complex polysaccharides, having strongly acidic side groups and calcium salts, to proteins, produces stronger fibres with increased water absorption and also fibres from solutions with low concentrations of proteins (Giddey, 1960). Hartman (1978) cites a process where hollow spun fibres are produced, which allows for centre filling with fats, gelatins or nutrients. Plasticized melt spinning using dry spinning extrusion technology, with subsequent microwave heating was patented by Coplan et al. (1976). Although a number of other processes have been patented, though not industrially used, of major concern to this research are the patents relating to the formation of a fibrous protein product by freeze texturization.

6.6 Freeze Induced Texturization

Structural modifications to proteinaceous material by freezing was first observed in sixteenth century Japan, with the production of kori-tofu by freezing tofu, a soybean protein curd, (Watanabe et al., 1974). This process was initiated as a means of preserving the tofu, though upon thawing, the product was found to have a stable, porous, sponge-like matrix, with voids where the ice crystals had formed. A subsequent dehydration step, producing a stable product at room temperature, did not significantly alter the structure, and produced an easily rehydratable product. Watanabe et al (1974) refer to this product as freeze denatured soybean protein.

Kori-tofu is presently being produced in large quantities in Japan. The soybean curd, tofu, is squeezed of excess moisture, frozen between -10 to -20°C and stored at -3°C for three to four weeks, (Smith and Circle, 1978; Arimoto and Sakuai, 1965). The storage period at several degrees below freezing is essential in this process to attain a stable product. This agrees with work by Finn (1932) and Moran (1934) who found the maximum degree of protein denaturation or insolubilization to occur with extended storage, 30 days, at near -3°C. Upon thawing, the kori-tofu is rolled of excess moisture and dried to approximately 10% final moisture content. This process, however, was not introduced as a specific means of texturizing the protein gel. Only one publication suggests any possibility of flavoring this material so as to have it resemble meat (Smith and Circle, 1978).

The first reference to freeze-texturization in recent literature was by Okumura and Wilkinson (1970) in their patent. A defatted protein extract from soybean or other vegetable material was frozen while in a slurried state. The fibrous nature of the resultant protein product, caused by ice crystal formation was noted. However, the primary reason for the freezing step was the separation and removal of non-palatable flavors associated with carbohydrates which remained entrapped within the crystal formation. The fibres were heat set upon thawing, but subsequently comminuted to make protein suitable for milk production.

Boyer and Middendorf (1975) were awarded the first patent for simulating a meat-like textured food product by freeze-texturizing protein concentrates and slurries. In the typically laudatory nature of patents, their "product has chewing and mouthfeel characteristics similar to those of natural meat products and can be substituted for meat in a wide variety of uses.... Dense proteinaceous areas having definite markings are highly similar to the well defined myofibrillar muscle tissue in natural meat such as red meat, fish and poultry." (Boyer and Middendorf, 1975). In a subsequent lengthy patent, Middendorf et al. (1975) expanded the generalities of the process, though discussed in only vague terms the effects of altering parameters such as total solids and freezing rates.

The general process outlined by Middendorf et al. (1975) is followed in several later patents by Noguchi et al. (1979), Kim and Lugay (1977, 1978, 1979) and Livingstone et al. (1979). Freeze-texturization, (also freeze-alignment, or freeze-fibre formation) is caused by the growth of ice crystals in a proteinaceous material. Until a eutectic point is reached, only water freezes or binds onto the crystal structure. The growth of the ice crystals forces the proteinaceous material ahead or to the side of the ice crystal boundary, causing a separation and compaction of the material, along with a dehydration effect. (Kim and Lugay, 1981).

A wide range of proteinaceous materials have been suggested as suitable for this process, (Kim and Lugay, 1981), although it appears that there have been limited investigations into possible differences between source materials. Kim and Lugay (1981) suggest that the main characteristics required of the material are good solubility and heat setting properties. They also state that the proteinaceous material may contain insoluble materials, though the tensile strength of the product is dependent on the concentration of non-solubles. However, they show no data to verify this.

Boyer and Middendorf (1975), Middendorf _et_ _al_., (1975) and Noguchi _et_ _al_. (1979) suggested using an alkali extracted, acid precipitated slurry, or a finely comminuted protein, (from soybean, animal or fish sources) dispersed in a water suspension. Middendorf _et_ _al_. (1975) suggest that the pH is not critical over a wide range, although the "optimal effect" will be found with a pH between 4 and 7. Kim and Lugay (1977, 1978, 1979, 1981) and Livingstone _et_ _al_. (1979) suggest that a protein solution will be more advantageous, as the proteins exist in an unfolded state which allows for greater protein-protein interactions in the freezing and subsequent heat-setting steps. They also state that weaker structures are produced with proteinaceous materials which are near the isoelectric point, due to reduced protein-protein interactions.

The patents suggest a wide range of possible solids concentrations (5% to 40% total solids) for suitable texturization, though there is little discussion as to the effects of these concentrations. Kim and Lugay (1981) state that a low solids concentration is desirable as the fibrousness decreases with increasing concentration. They also suggest that low protein concentrations result in a rather loose texture, whereas a high protein concentration gives a tight fibrous texture. However, no data are shown to support this.

Use of a single planar freezant/protein interface is suggested to produce generally unidirectional fibres, normal to the freezant surface. A semi-infinite slab design was used in all but two patents. Noguchi et al., (1979) used non-directional freezing, and Middendorf et al. (1975) suggested the use of semi-infinite cylinders. The ice crystals were found to grow normal to the freezant/ protein interface, forcing the proteinaceous material ahead of the ice crystal boundary, thus producing orderly fibrillar formation. These distinct fibres were found to be connected by various points of protein cross-linking, sufficient in numbers to form a cohesive fibrous mass. The reason for these cross-linkings is not discussed.

The freezing rate was found to have an effect on the size and number of ice crystals and consequently the fibre size and numbers. Fast freezing rates produced smaller, more numerous crystals and fibres, while slower rates produced larger, less numerous crystals and fibres. This was stated in all patents. Freezing rates, defined by phase boundary advancement, were suggested to range between 0.03 to 0.5 ft/hr (Kim and Lugay, 1981). Fissures perpendicular to the ice crystals could be produced by extreme cold shock treatment, such as immersion in liquid nitrogen (Kim and Lugay, 1979).

Removal of the water and heat setting of the protein fibres can be incorporated into one step,(Boyer and Middendorf,1975; Middendorf _et al_., 1975). Rapid heating, usually by steam, reduced the time between the water phase change and the irreversible heat setting of the protein fibres. This was considered mandatory to retain the fibrous structure.

Kim and Lugay (1978, 1979, 1981) claimed that heat setting of the frozen material with a high water concentration produced a poor quality material with a substantial loss of the fibrous structure. The solubilized state of the protein solution that they used, prior to freezing, might have affected a more rapid loss of fibre structure upon thawing, as compared to the precipitated protein slurry. The frozen material therefore required some method of partially setting and removing water from the fibres. A freeze-drying process or ethanol/water substitution step was suggested prior to thermally setting the material. However, these processes are both expensive, and in the freeze-drying case, very energy intensive. Subsequent acidification steps were also required to return the pH to that of post rigor meat. Kim and Lugay (1981) also made no mention of possible detrimental effects of prolonged alkaline exposure of the protein.

Two groups of Japanese researchers, Noguchi et al.,(1979) and Hashizume and Watanabe,(1975), were awarded patents involving the use of freezing of a proteinaceous material. Hashizume and Watanabe (1975) investigated the effects of the addition of a divalent salt, CaCl , to the protein solution prior to freezing, though the work dealt primarily with protein extraction. Noguchi et al. (1978, 1979), produced a material similar to Kori-tofu, (a sponge-like protein matrix) using alkaline extracted, acid precipitated proteins from Krills. This was the only work in which an objective textural measurement was attempted, using an undefined rheometer for a tensile strength analysis. The general trend showed that slow freezing,(-7.5°C) produced a higher tensile strength than fast freezing (-25°C). The gel strength was found to be highest at pH 7. The addition of NaCl or CaCl to the material was also found to increase the gel strength. Noguchi et al. (1979) also found that bubbling oxygen through the precipitate for several minutes increased the tensile strength over three fold, yet other patents suggest that a deaeration step is preferable (Middendorf et al., 1975; Kim and Lugay, 1978, 1979, 1981). The deaeration step was to prevent the formation of small air bubbles which could deform the fibres. Noguchi et al. (1978) suggested that the addition of oxygen would effect possible thiol-disulfide reactions.

Possible biochemical reactions occurring during the freezing process, particularly involving protein-protein interactions, have been suggested by various researchers. (Levitt, 1966; Connell, 1968). Hashizume et al. (1971, 1974a, 1974b) suggested possible reactions that may occur during the formation of kori-tofu. However, the biochemistry of freeze-texturization is beyond the scope of this work.

6.7 Ice Formation

The formation of protein fibres by freeze texturization is directly dependent on the formation of ice crystals and the factors affecting the ice crystal nucleation and growth rate. As only water freezes until the eutectic point is reached, the proteinaceous material is forced to leave the areas in which the ice crystals form, being pushed ahead and to the side of the advancing boundary. At the same time, the proteinaceous material is being dehydrated by the freezing out of the water.

The size and number of fibres is directly proportional to the size and number of ice crystals. Therefore, the factors affecting the formation of ice crystals must be understood to determine the manner in which they affect the protein fibre formation. Ice crystal formation occurs in two separate stages; crystal nucleation and crystal growth.

6.7.1 Nucleation

In pure water, with no foreign particles and the absence of ice, supercooling to -39°C can occur before homogeneous nucleation takes place (De Quervain, 1976). However, with foreign particles in solution, the degree of supercooling is greatly reduced, sometimes to the point where it is non-discernable, causing heterogeneous nucleation. This is suggested to be caused by the catalytic effect of the particles on nucleation, as the particles may have a surface configuration similar to ice crystals, or the bound surface water may be ordered to the extent of acting as a base for crystal growth (Meryman, 1966). The latter effect would be the probable cause for limited supercooling in a proteinaceous slurry.

The probability of nucleation occurring is dependent on the degree of supercooling, the number and type of particles present, and the rate of energy removal from the system. Fig. 10a shows the general trend observed as the energy is removed from a water system. The size, number and type of foreign particles found in the solution will effect a horizontal shift of the curve. Any factors increasing the possibility of nucleation, i.e. increased number of particles, or increased ordering of the surface water molecules, will cause a shift to the left (Fennema, 1973).

6.7.2 Crystal Growth

The general trend for the rate of growth of ice

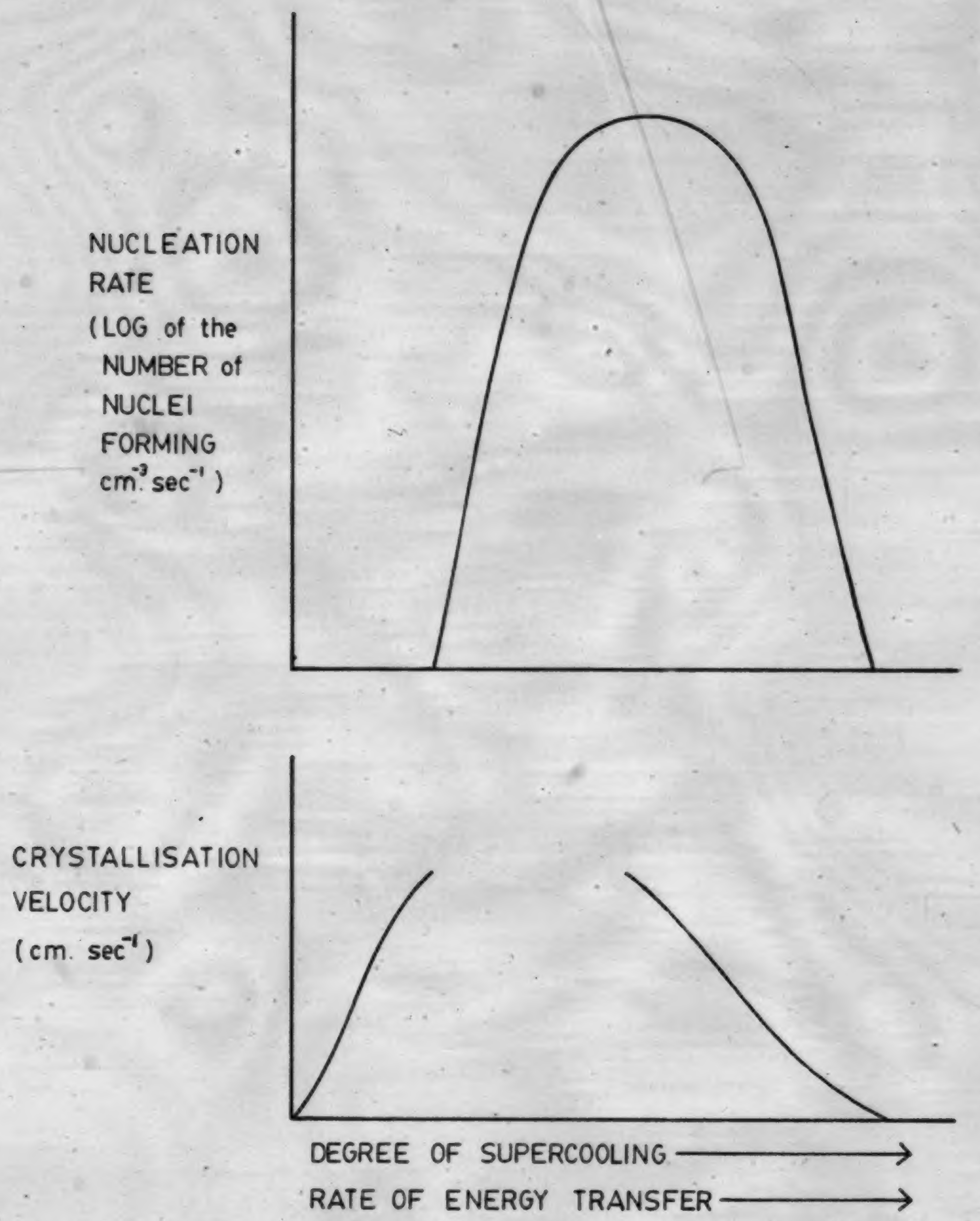


Figure 10 Nucleation Rate and Crystallization Velocity vs. Rate of Energy Transfer.

(from Hallett, 1968, & Fennema, 1973)

crystals is shown in Fig. 10b (Fennema, 1973; Hallett, 1968). The crystal growth rate is dependent on the rate of energy and mass (water) transfer through the material, along with the rate at which the water molecules are aligned to fit into the crystal lattice (Hallett, 1968; De Quervain, 1976). The mass transfer and molecular rearrangement are dependent on the viscosity and moisture content of the material. Altering these and other parameters can have the effect of horizontally shifting the curve in Figure 10b or changing the maximum rate. Luyet, (1966, 1968) investigated the effects of altering these parameters on the size, number and type of crystals formed.

Luyet (1968) found a hexagonal planar crystal growth when the cooling rates were slow enough to allow the water molecules to be positioned into the crystal lattice. The crystals have three axes at 60° to each other, with secondary branches forming at 60° to the primary branches. As only water freezes initially, the intercrystalline spaces contain a solution with an increased concentration of solutes and solids.

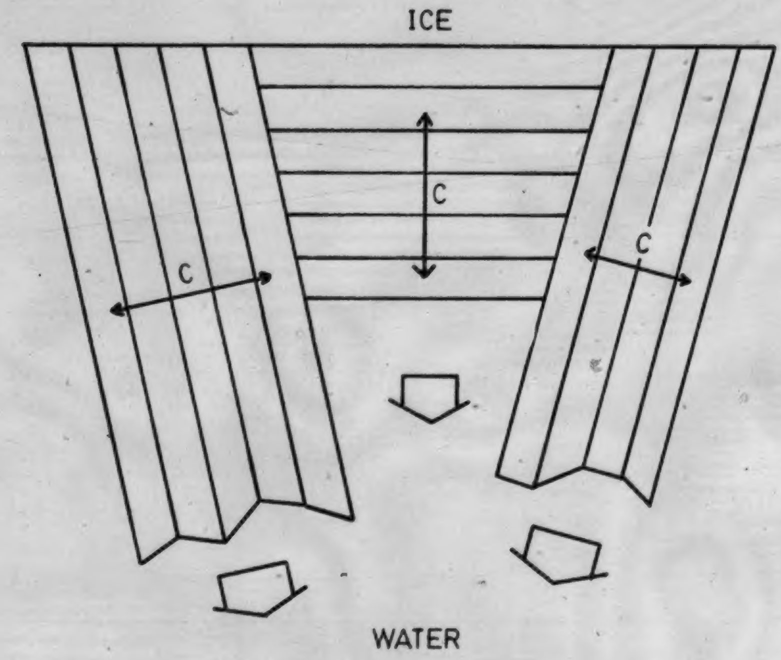
As the cooling rate is increased, the time available for the water molecules to form an ordered structure is reduced. This causes an increase in the number of axes or "spear" crystals formed from the nucleation centre, with reduction in secondary 60° branching from the primary branches. The greater the rate, the greater the number of irregularities from the hexagonal form, and the greater the number of primary branches. These are classed as irregular dendrites, and at greater rates, spherulites (Luyet, 1968). The irregular dentrites have some secondary branching while the spherulites exhibit very few. Increasing the cooling rates within either the hexagonal or irregular dendrite classifications was found to cause an increase in splitting of the branches. The irregular dendrite is the most commonly encountered crystal formation found in frozen food materials, as the size of the materials usually prohibit a rapid enough cooling rate to form spherulites, and the tissue structure can inhibit hexagonal growth.

As shown in Fig. 10 a & b, decreasing the temperature and increasing the cooling rate causes an increase in the number of nucleation sites and a decrease in the crystal volume. Inversely, decreasing the cooling rate causes a decrease in the number of nucleation sites, with increased crystal volumes. Both the crystal growth rate and nucleation rate reach a maximum point and decrease with increased cooling rates, though large sample sizes would prohibit a rapid enough energy removal to allow this maximum to be reached.

Ice crystal growth occurs most rapidly in a planar direction, defined as the a axis, while to a much lesser extent on the c axis (Hallett, 1968). The rate of growth appears dependent on the molecular topography of the crystal surface, with rough surfaces (a axis) being more preferred than smooth surfaces (c axis). For this reason, crystals with the c-axis parallel to the cooling interface, grow more rapidly and encroach on those crystals growing with their c axis perpendicular (Fig. 11) (De Quervain, 1976). Therefore, ice crystals growing normal to the cooling interface are more preferred.

Luyet (1968) found that an increase in the solute concentration caused a decrease in the number of crystal lobes or branches, thereby increasing their size and the size of the interstitial area. Increasing the solute concentration also appeared to increase the number of nucleation sites, causing a decrease in the overall crystal size. The increased solute concentration would cause a reduction in the water diffusion rate and the mobility of the water to align for correct binding. It could also cause a physical obstruction to the crystal. Although the increased particle content may also increase the nucleation rate, it is more probable that the inhibition of crystal growth would cause an apparent, though not real, increase in the nucleation rate, which would account for the increased number of nucleation sites.

As the ice crystals grow into a media, the layer of non-frozen material on the ice front boundary increases in concentration of both solutes and solids, thereby reducing its freezing point. Under conditions of 0°C temperature equilibration , rapid freezing, and reduced diffusion of solutes, Taborsky (1979) found that it was possible for nucleation to occur in advance of the freezing boundary.


ICE
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WATER

Figure 11 Crystal Growth vs. Crystal Orientation.
(from de Quervain, 1975)

This leap frog effect could lead to pockets with high solute concentrations within the ice structure, plus a break of the ice crystal network, (Love, 1966), causing a disruption of any orderly fibre structure. Meryman (1966) found that ice crystals grow preferentially in areas of lowest solute concentration, caused by the obstructive nature of solute build up and its depressed freezing point. Therefore, any solute build up will inhibit crystal growth in that direction.

The rate of energy transfer is the major factor affecting the size, number and type of crystals formed. Therefore the geometry and the thermal properties of the freezant protein interface have a profound effect on the crystal structure. Meryman (1966), investigated the differences between crystal growth in a semi-infinite slab, a semi-infinite cylinder, and a sphere with a 7% starch solution, (Fig. 12). The cylindrical and spherical models showed a more rapid drop in temperature following the start of freezing than did the slab. Also, the crystal size at the centre of the cylinder and sphere were particularly small, whereas the slab crystals increased in size with increased distance from the protein/freezant interface.

As the freezing boundary advanced through the material, the rate of energy removal at the phase change interface decreased due to the reduced energy transfer through the frozen section as compared to the energy transfer at the material freezant interface. This would reduce the freezing

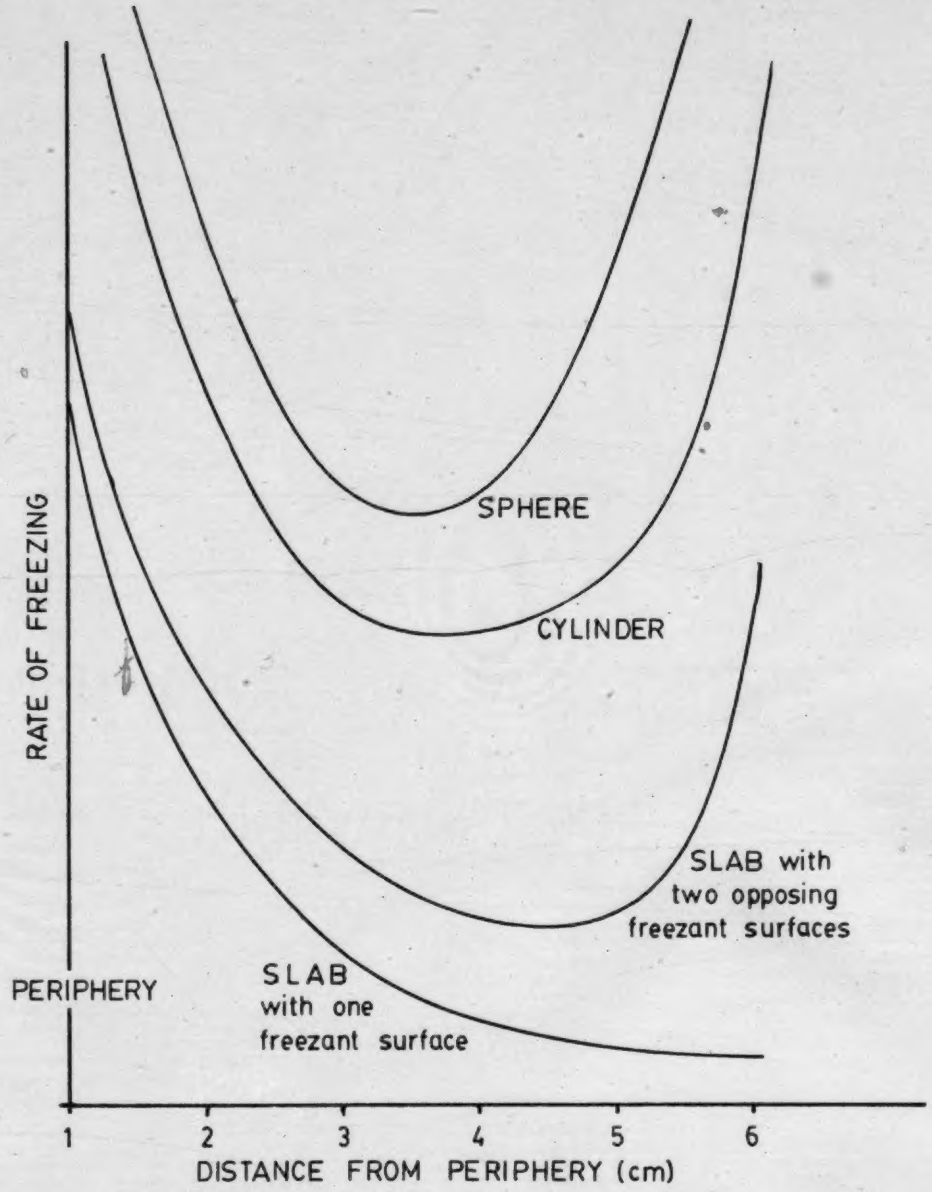

Figure 12 Effect of Freezing Form on Rate of Freezing with Respect to Distance from the Periphery (with a 7% Starch Solution). The curves have been vertically displaced to simplify visual comparison.

(from Meryman, 1966)

rate with increased distance with an infinite slab geometry. However, the cylinder and sphere both show an increase in freezing rates towards the centre. This is due to the change in surface to volume ratio (2/r for a cylinder, 3/r for a sphere) therefore there is a decrease in the ratio of unfrozen volume losing energy to the surface area of the freezing interface. This accounts for the smaller crystal size and the more rapid temperature drop.

Meryman (1966) suggests that the determination of the freezing rate be made from the falling temperature zone during or immediately following freezing. The extent of the plateau (time) at slightly below 0°C was found to have no effect on the crystal size; this was strictly a period of temperature equilibration prior to freezing. The best correlation with crystal size was found to be a combination of the rate of temperature drop and the rate of boundary advancement.

6.7.3 Recrystallization

Due to the unstable nature of ice crystals, modifications to the crystal structure,(recrystallization), may occur after freezing. Burgers (1963), defines recrystallization as any change in the number, size, shape, orientation, or perfection of crystals following initial solidification. Fennema (1973) lists five types of recrystallization; Iso-mass, Pressure Induced, Irruptive, Migratory, and Accretive, though it is likely that only the latter two types occur predominantly in foods. The rate of recrystallization was found to be dependent on the temperature, (ie. higher sub-zero temperatures caused a greater rate of recrystallization), and the storage time, (Dowell and Renfret, 1960). Recrystallization predominantly involves an increase in the average crystal size with a decrease in the number of crystals. Therefore, as the temperature of the frozen material increases (still sub-zero), there is an increased probability that the larger crystals will expand more rapidly at the expense of smaller crystals. The numerous small crystals formed during rapid freezing then have a reduced stability at higher temperatures.

Recrystallization has been shown to occur more rapidly in pure water than in a biological matrix which can inhibit moisture transfer and prevent crystal growth development. Luyet (1939) and Luyet et al. (1966) found that samples with large molecules such as protein or starch, required higher temperatures before recrystallization occurred, than did samples with smaller molecules, ie. sucrose. Most research on recrystallization in a biological matrix has shown that relatively high sub-zero temperatures or extended storage periods with initially unstable, rapidly frozen small crystals were required to cause a large degree of crystal enlargement. Luyet and Gibbs (1937) showed that crystal growth or enlargement occurred at -4 to -8°C in rapidly frozen plant cells after several hours. Menz and Luyet

(1961) found some crystal enlargement in rapidly frozen muscle fibres when the temperature increased to -15°C. In both cases, the temperature had to be increased dramatically. Therefore, the extent of recrystallization that may occur in a matrix with a relatively high protein content which is stored for a short period, at a temperature near or below that which it was frozen, is very minimal.

6.7.4 Physicochemical Changes

Physicochemical changes occur in the freezing process, caused primarily by the increase in concentration of solutes and solids, a reduction in temperature and the physical structure of ice.

As the ice crystals grow, the quantity of water removed from the non-frozen material increases. Finn (1932) determined that close to 80% of the water in an ox muscle protein solution was frozen by -5°C, while 90% was removed by -10°C. Although the amount of water frozen is dependent on the initial concentration, Meryman (1966) showed that the percentage of water frozen at the same temperature was similar with different materials. Kuntz (1979) found a final concentration of 0.3 to 0.5 gms water/gm protein when a dilute aqueous protein solution was frozen down to -10°C. This remained essentially constant with a further reduction in temperature. The extent to which the water is initially bound within a system will strongly affect the percentage frozen and therefore the final concentration.

Ice crystal formation also causes an increase in the volume occupied by the liquid phase. Pure water exhibits a 9% volume expansion when frozen at 0°C, with a small degree of contraction occurring upon further cooling (Fennema, 1973). This change in volume has then to be accounted for by the expansion of the sample, or, if possible, to a lesser extent by a compaction of the solutes and solids in the unfrozen phase.

This dehydration and reduction of the water activity, along with the volume increase, will affect, to varying degrees, the properties of the unfrozen stage and induce possible interactions between the solids, solutes and water. Changes in the pH, ionic strength, viscosity, freezing point, and oxidation reduction potential are likely to occur. In a protein solution or slurry, the proteins are forced closer together, due to the removal of water and the compacting influence of the crystals, causing an increased possibility of intermolecular interactions (Fennema, 1973).

Van den Berg (1968) did extensive research on the effects of freezing and frozen storage on physicochemical changes in foods. The pH of the unfrozen phase was found to fluctuate as the water concentration decreased, caused by increased salt concentrations and salt precipitation. Poultry was found to increase slightly in pH from pH 5.7 (post rigor) to pH 6.0, when frozen at -10°C (Van den Berg, 1968). Finn (1932) found a slight, steady decrease in the pH of frozen muscle juice as it was frozen down to -6°C, after which there was a rapid rise to the initial pH or slightly higher.

As essentially all of the salt remains in the unfrozen phase, the increases in salt concentration were found to be inversely proportional to the amount of water remaining in the unfrozen phase, until a eutectic point is reached, (Van den Berg, 1968). The ionic strength increases proportionally. Therefore, dependent on the extent of the concentration of the salt, a salting in or salting out effect will occur, causing some protein dissolution or a further loss of water from the protein (Anglemier and Montgomery 1976; Young, 1975).

Possible protein-protein interactions, including thiol-disulfide interactions, have been hypothesized as the source of protein denaturation by freezing in plant proteins (Levitt, 1966) and in Kori-tofu (Hashizume _et al_., 1974a, 1974b), though there is still some disagreement as to this mechanism. Effects of freezing temperature on protein denaturation were investigated by Finn (1932) on ox muscle juice (Fig. 13) and Moran (1934) on a beef protein solution. A maximum protein denaturation at -3°C with a 30 day storage time was found, with a rapid decrease with either a rise or drop in temperature. Tests run with a shorter storage periods showed a greatly decreased amount of protein denaturation (Fig. 13). The degree of denaturation was dependent on the solubility of the protein. Finn (1932) attributed the high denaturation to the increased acidity

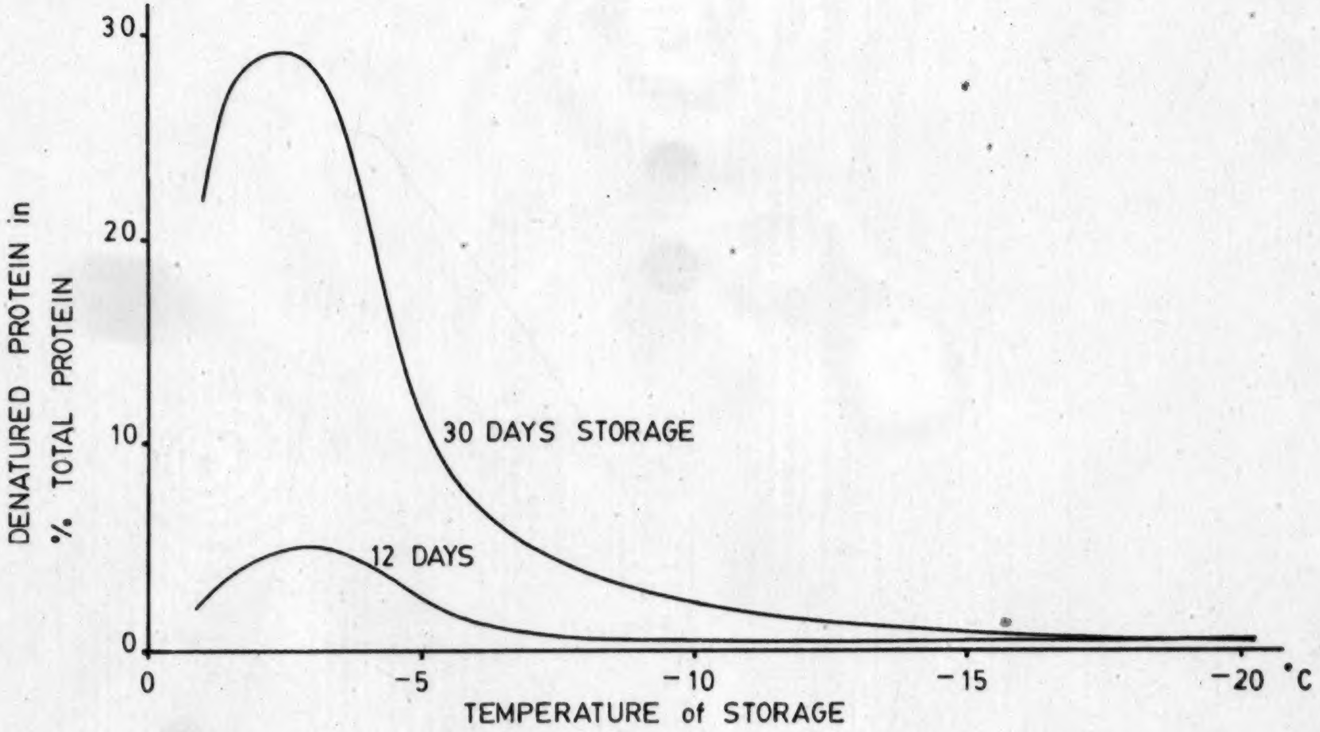

Figure 13 Protein Denaturation of Ox Muscle Juice vs. Storage Temperature and Time.

(from Finn, 1932)

and increased ionic strength at this temperature and unfrozen water concentration.

The dramatic change in the extent of protein denaturation and its reaction rate with small temperature increments can be explained by the change in concentrations in the unfrozen phase and the temperature decrease. The solute/solids concentration increase can cause both an increase or decrease in reaction rates, dependent on the system. However, a temperature decrease is most likely to cause a decrease in the reaction rates (Fennema, 1973). Therefore, at temperatures slightly below the freezing point, ie. -5°C, where a large increase in concentration has occurred, this concentration effect may predominate over the temperature effect. Further concentration may cause a decrease in reaction rates due to a decreased solutes/solids mobility. The temperature reduction would combine with this to cause a rapid drop in denaturation rates, (Connell, 1968).

The sarcoplasmic proteins have been shown to be relatively stable to any reactions caused by freezing (Dyer and Dingle, 1961; Connell, 1968). Electrophoresis failed to reveal any changes caused by frozen storage. Awad et al. (1968) however, attributed the change in the percentage of total extractable protein,(91% to 51% for beef stored at -4°C) to both the sarcoplasmic and myofibrillar proteins. In a test on frozen storage of chicken, Hamm, (1970) showed that the free amino acid content increased remarkably at

-3°C after two months, though at -28°C, almost no changes were detectable. However, it was not specifically stated which protein fraction was affected. Modifications to the myofibrillar proteins in fish muscle have been found, though a review by Connell (1968) showed that most of these results were based entirely on protein solubility and extractability in neutral salt solutions. This change in solubility was found to be affected by other aspects of processing also, so the actual extent of freeze induced "denaturation" is not known. However, it has been shown that the myofibrillar fraction is more susceptible to this "denaturation" than the sarcoplasmic fraction.

The limited research on freeze induced texturization has illustrated that some degree of texturization can be imparted in a protein in this manner. The majority of the research that has been covered in this field, has been only recently published while this research was in progress. However, as most of these are patents, only general descriptions of the processes and the products are available. Therefore, the parameters affecting the freeze texturization of protein have yet to be investigated.

7. Methodology

The protein precipitate produced by methods described in Part 1 was used as the raw material in all freezing studies. The precipitate was kept at 4°C prior to usage.

To produce the freeze induced texture the samples were frozen in semi-infinite cylinders in all experiments except for those described in section 7.1, where a semi-infinite slab was also utilized. The samples were frozen in a Liquid Carbonic Cryogenic Freezer, using a liquid carbon dioxide freezant, at a preprogrammed temperature. Once frozen, the samples were sealed in plastic bags to prevent loss of moisture and stored at -25°C for two days. The frozen samples were then heat set, using a Toshiba Microwave Oven (Model ER-786BT, 650 W), for approximately six minutes or until the internal temperature reached 85±5°C. Non heat set samples were thawed at room temperature in a sealed container to prevent moisture loss. All samples were kept in sealed containers at 4°C until further analyzed. Certain modifications to this general procedure were employed for specific purposes as indicated below.

7.1 Freezing Form

A semi-infinite cylinder design was produced using 5.4 cm diameter sausage casings. Wood corks of a similar diameter and a 3.5 cm thickness were placed at each end of the cylinder to restrict axial heat flow. The casings were filled with the precipitate to a length of 15 cm. Cylinders with 2.4, 4.0, and 9.0 cm diameters were made similarly. These were filled slowly to reduce the number of air pockets. The cylinders were tied at each end and hung in the freezer.

A semi-infinite slab was made by cutting a 2.5 cm deep rectangular box in a 10 cm deep block of styrofoam. The edges were insulated with 5 cm of styrofoam. The top surface was left uninsulated. This slab was filled with the protein material and placed in the freezer.

Changes in the fibre structure due to the freezing form were determined by measuring the differences in total solids between the half of the sample which contacted the freezant and the half which was insulated from the freezant. One centimeter thick discs, cut from the middle section of the cylinders, were cut in the centre using a cork borer whose radius was half the cylinder's radius, producing halves of different areas but similar radial thickness. The halves from the slab and cylinder forms were therefore cut at a similar distance from the protein/freezant interface.

7.2 Material Source

The protein precipitate used for freezing was prepared in one of the following three ways, dependent on the experiment.

a. The alkali solubilized protein (see Part 1) was precipitated at pH 4.5, 5.0, 5.5 or 6.0 with hydrochloric acid as described in Part 1. The slurry was centrifuged at 4,000 rpm in a J-21 Beckman centrifuge, with a JA-14 rotor.

b. The alkali solubilized protein was precipitated at pH 4.8, 5.5 and 6.2 with hydrochloric acid. This slurry was centrifuged at 2,000, 3,500, 5,000, 7,500 rpm in a J-21 Beckman centrifuge with a JA-14 rotor. The high protein solubility associated with pH 6.2 samples required a further centrifugation at 9,000 rpm to sufficiently reduce the water content.

c. The alkaline solubilized protein was precipitated at pH 4.5, 5.0 and 6.2 using acetic, hydrochloric, phosphoric or sulfuric acid. This was centrifuged at 4,000 rpm in the previously described centrifuge.

7.3 Determination of Freezing Boundary Movement

Samples (5.4 cm diameter) were frozen in a temperature controlled Liquid Carbonic cryogenic freezer using liquid carbon dioxide as the freezant. The rate of the frozen boundary movement was determined by placing four equally spaced thermocouples along the radius, with one at the centre and one on the surface. The thermocouples were placed in a spiral, to prevent interference between each other, at the centre of the length of the cylinder.

7.4 Photography

A photographic record was kept of the effects of the process parameters on the fibre structure. Thin 5 mm slabs were cut using a sharp razor, from the central region of the heat set samples after they had cooled to room temperature. It was imperative that the samples had a consistent flat surface after cutting to ensure complete focusing across the surface. This is due to the limited depth of focusing field which is inherent to macrophotography. Pictures were taken on Kodak, Ektachrome 160 ASA Tungsten slide film using a rigidly mounted 35 mm Canon EF camera. Magnifications of 1:1 were taken using a 50 mm, F3.5 macro lens with a 2.5 cm extension tube. Magnifications of 4:1 were taken using a 24 mm F2.8 lens which was reverse mounted and combined with the 2.5 cm extension tube. Maximum depth of focusing field was obtained by using high aperture F values between F16 and F32 where possible. Some color shifting occurred on the slides due to the lighting and the extended times required and some incompatability with the lighting used.

The slides were printed using a positive printing process (Kodak R1000 chemistry on 2203 paper) to minimize darkening and loss of detail. Final print magnifications of 7:1 and 28:1 were obtained from the 1:1 and 4:1 initial magnification slides respectively.

7.5 Retort Stability

Six semi-circular samples, from 5.4 cm diameter cylinders, weighing approximately 12 grams each, were cut from the pH 4.8, 5.5 and 6.2 samples frozen at -10 and -25°C and subsequently heat-set. These were placed in 200 ml cans filled with a heated (80°C) 2% brine solution, sealed and retorted at 121°C. Retort times were 0, 10, 20 and 60 minutes. Samples were removed and stored at 4°C for textural analysis. The brine was filtered to determine the weight of particles broken from the samples.

7.6 Textural Analysis

Identical size samples 1.0 x 1.0 x 2.5 cm were cut from 1 cm thick discs from the central region of the cylinder. This was the maximum size possible for the load cell used. Two samples were taken from each disc. Weighed samples were then placed in a Kramer Shear Cell (10 blades) with the majority of the fibres parallel to the blades. The cell was placed in an Instron Universal Texture Testing Instrument with a crosshead speed of 8 cm/min. The maximum or peak force was determined from a strip chart recorder, subtracting the frictional force of the blades from the apparent peak force.

The samples were cut as close to the same size as possible, using a template. However, small variations occurred. Szczesniak et al., (1970) showed that over small changes in weight, the maximum force was a linear function of the weight. As these samples were of similar dimensions, and approximately the same weight, the peak force was determined per unit weight, which accounted sufficiently for any variations.

The force-deformation curve for a Kramer Shear cell has been suggested to be a result of tensile, compression and shear forces (Voisey, 1976). However, as it has not been given a name, it is referred to strictly as the peak force in this work. The slope prior to the break point was not utilized.

The samples were run in quadruplicate.

7.7 Fibre Density

Three 5 mm thick discs were cut adjacent to the discs used in the texture measurements. Photographs were taken of each side of the disc at a 1:1 or 4:1 magnification with a 35 mm camera, using Kodak Ektachrome 160 ASA Tungsten slide film.

The 1:1 magnification slides were projected onto a screen which had six straight lines, of equal length, drawn at angles to each other. The direction of these lines were arbitrarily chosen so as to perpendicularly bisect as many fibres as possible. Two lines were chosen which were closest to being perpendicular to the fibre direction. The number of fibres crossing these lines was recorded, twice per slide, for four slides, and the fibre density per unit length determined.

7.8 Total Solids

Three 7.5 mm thick discs were cut from the central region of the heat-set cylinders and used for total solids analysis. Approximately 10 grams of non-heat set samples were also analyzed. Total solids were determined by placing the samples in a vacuum oven at 80°C for 24 hours.

8. Results and Discussion

8.1 Fibre-Formation

The formation of protein fibres is directly dependent on the ice crystal formation, and therefore on any factors influencing crystal nucleation and growth. These can be broken into two interdependent areas; heat transfer, through the frozen and unfrozen protein media, and from the surface to the freezant material; and mass transfer, primarily of water through the protein media. The properties of the fibres are dependent on factors affecting the ice crystal formation, the nature of the proteinaceous material prior to freezing and any secondary processing such as heat setting.

The large sample size and the nature of the water in the protein precipitate, ie. the organized bound surface water, is likely to limit the degree of supercooling that may occur within the sample to only a fraction of a degree centigrade. Furthermore, the small dry ice particles sprayed out by the freezer, would initiate crystal growth as soon as they contacted a sufficiently cooled sample. Therefore, the number of nucleation sites and the rate of crystal growth is strictly dependent on the rate of energy removal and not the degree of supercooling as shown in Fig. 10.

8.1.1 Time/Temperature Profiles

The time/temperature profiles for the 2.7 cm radius cylinders frozen at four different rates (ambient temperatures of -10, -25, -45, and -75°C) are shown in Fig. 14 to 17. The curves show temperature-time relationships at four equally spaced points along the radius, including one on the surface and one at the center. Meryman (1966) suggests that the temperature plateaus are strictly a temperature equilibration prior to freezing. This plateau was found to occur at -0.5°C.

The time at which the freezing boundary reaches the thermocouple is shown as the point at which the curve departs from the plateau. It is difficult to determine the precise time at which this occurs as the curvature is initially very gradual. Furthermore, the temperature is likely to remain at the freezing point for a short period after the freezing boundary reaches that site, due to the release of the latent heat of fusion. In this case the time lag between freezing and a drop in temperature is assumed consistent throughout each sample. (In reality, the time lag will vary, dependent on the amount of energy released and the rate at which it is removed).

The time at which the freezing boundary reaches the recording points in the cylinder, with respect to each freezing rate, is shown in Fig. 18. The increasing y-intercepts are due to the increased time required to reduce the surface temperature to the freezing point. The curves are shown as being initially linear, with a decrease in slope as the freezing front approaches the centre. In fact, due to the decrease in the rate of heat transfer

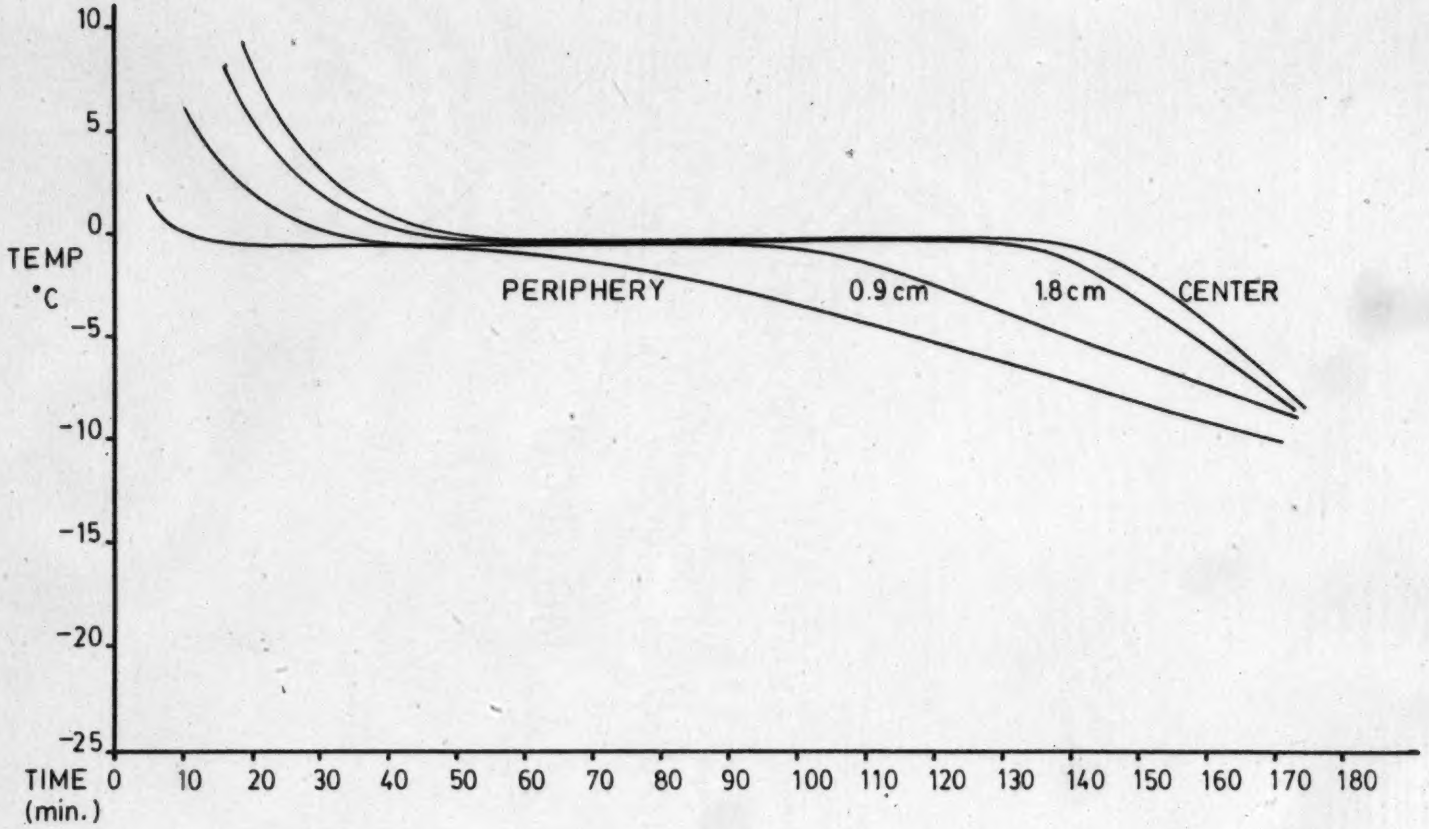

Figure 14 Time, Temperature Profile of 2.7 cm. Radius Cylinder with pH 5.5 Precipitate Frozen at -10° C.

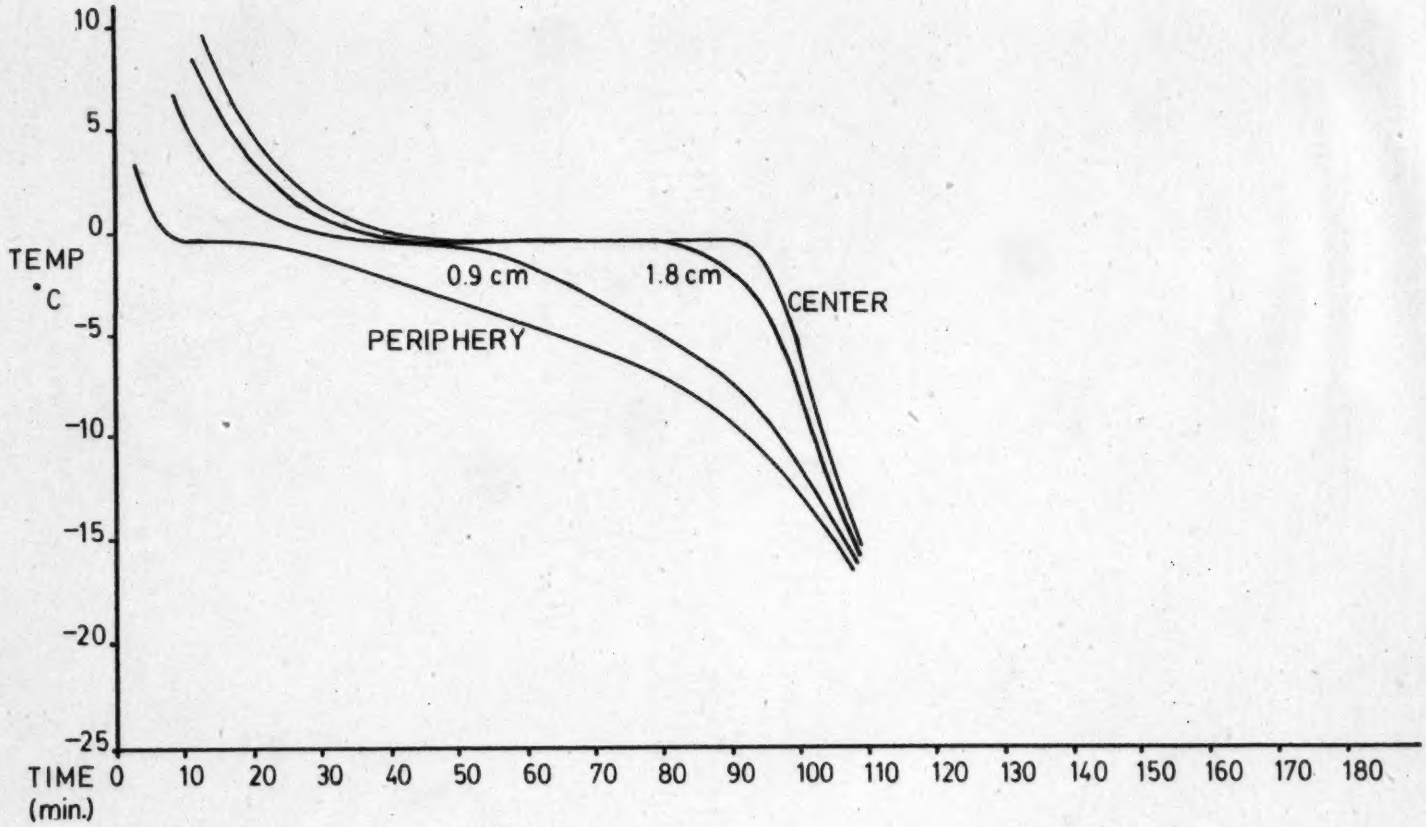

Figure 15 Time, Temperature Profile of 2.7 cm. Radius Cylinder with pH 5.5 Precipitate Frozen at -25° C.

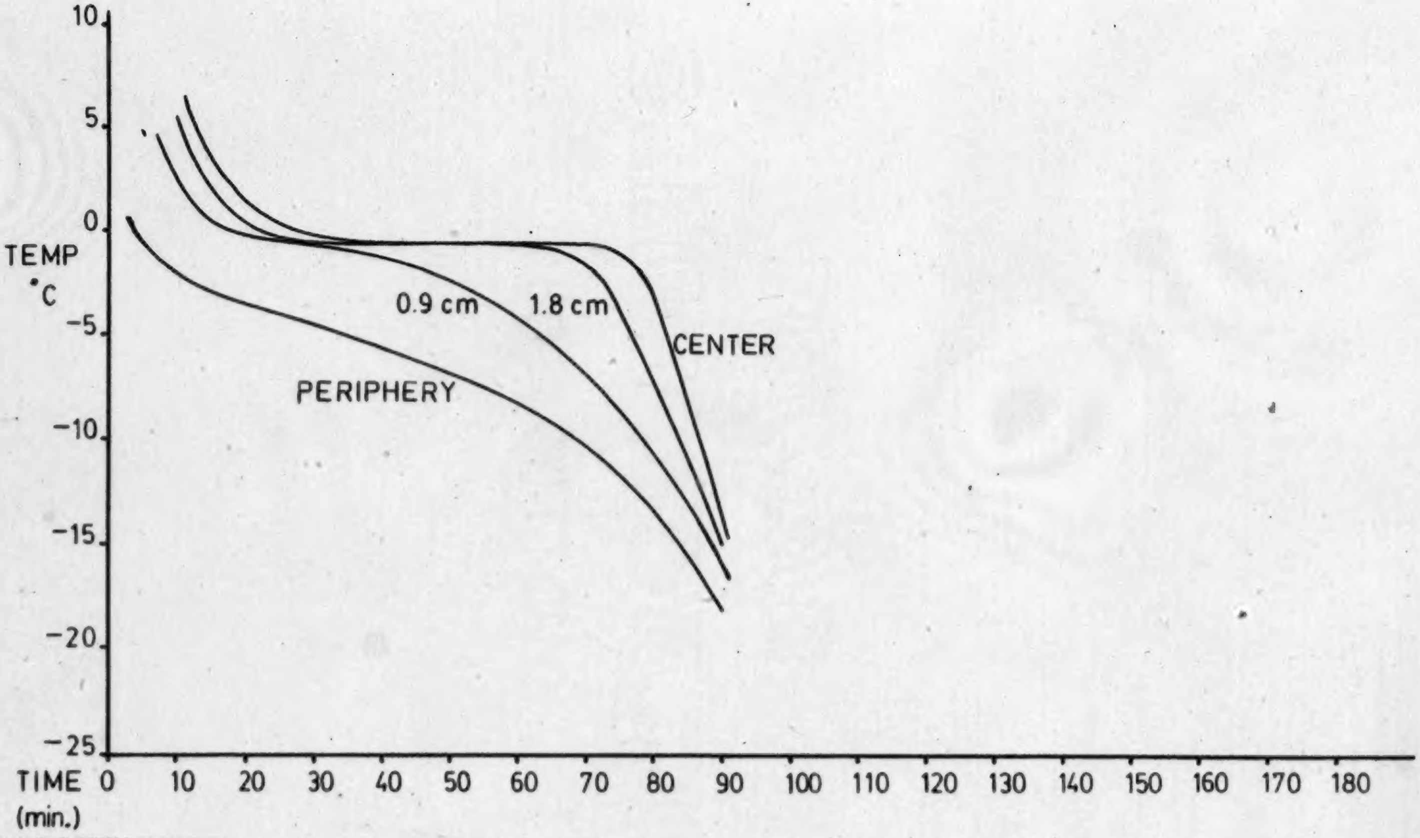

Figure 16 Time, Temperature Profile of 2.7 cm. Radius Cylinder with pH 5.5 Precipitate Frozen at -45° C.

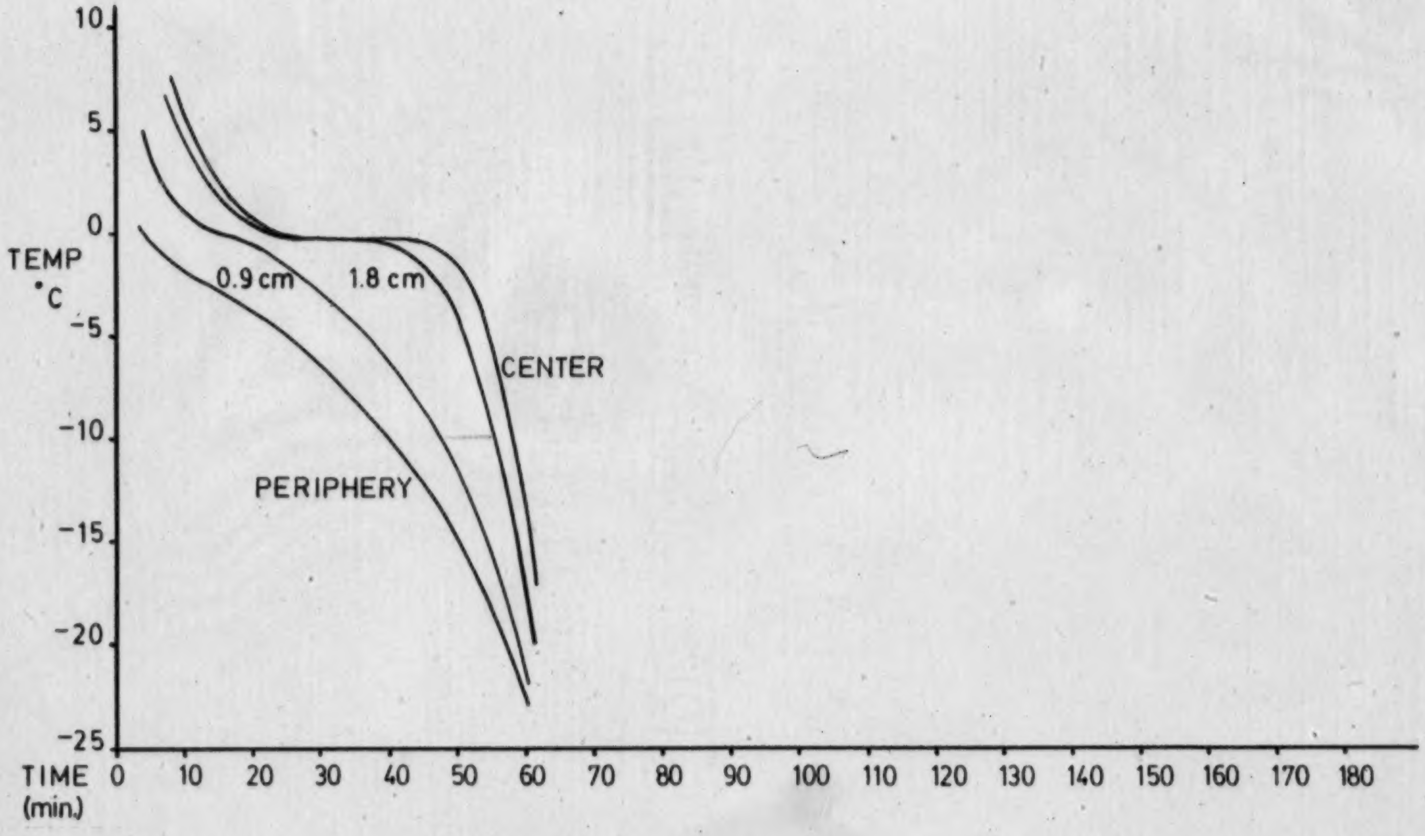

Figure 17 Time, Temperature Profile of 2.7 cm. Radius Cylinder with pH 5.5 Precipitate Frozen at -75° C.

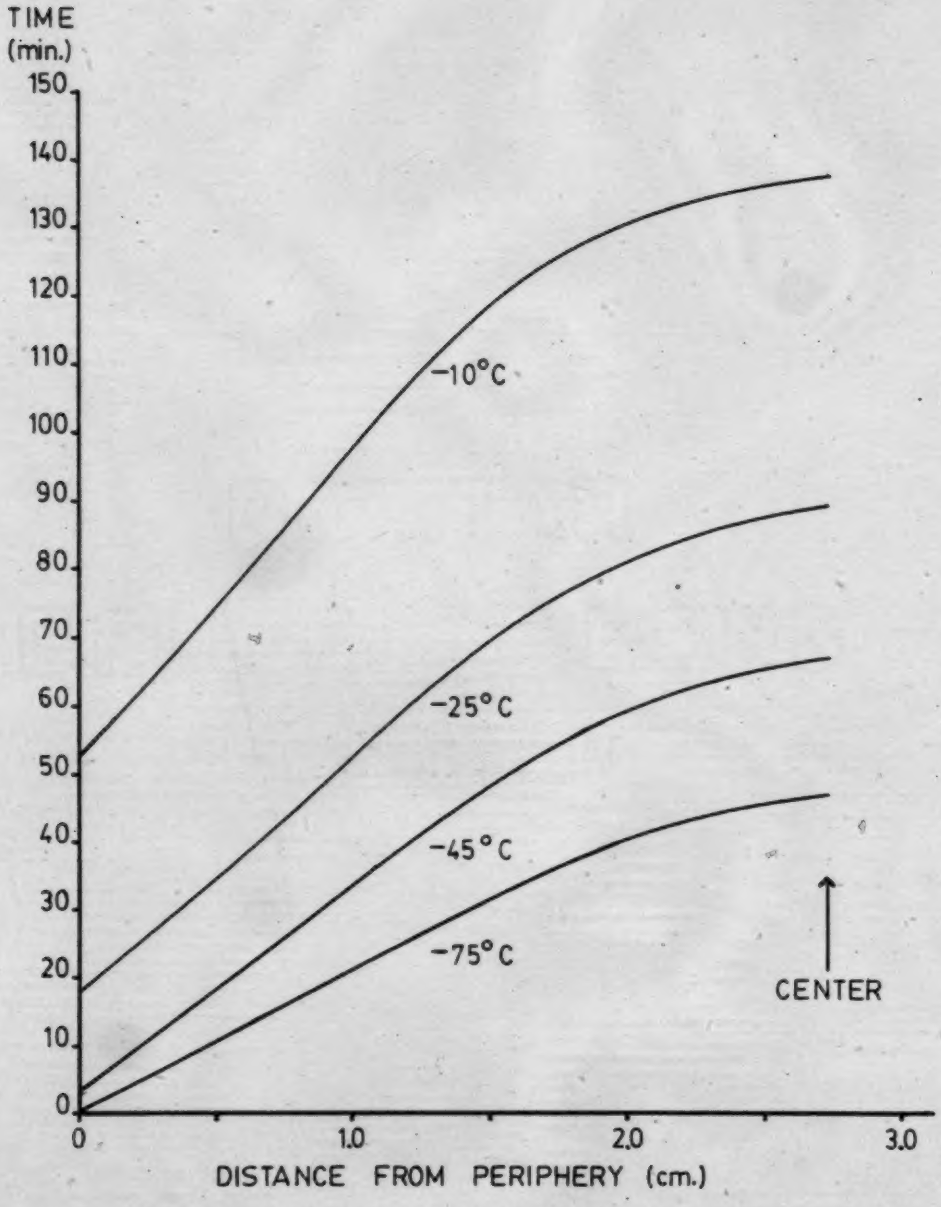

Figure 18 Frozen Boundary Movement vs. Freezing Temperature with a 2.7 cm. Radius Cylinder.

caused by the increased distance between the frozen boundary and the freezant protein interface, the curves are likely to be slightly "S" shaped.

The rate of heat transfer is a linear function of the temperature gradient between the freezing point and the ambient temperature. Therefore, lower ambient temperatures increase the rate of the frozen boundary movement, as shown by the decrease in slope. At the same time, the change in ambient temperature from -10°C to -25°C shows a greater change in the rate of the frozen boundary movement than decreasing from -25°C to -45°C or -75°C. Decreasing the temperature to -25°C from -10°C increases the gradient by 150%, whereas decreasing the temperature to -45°C from -25°C increases the gradient by only 80%. Therefore, the fibre characteristics which are affected by the freezing rates will exhibit a greater change between -10°C to -25°C than from -25°C to -75°C. This is discussed in greater detail in Section 8.3.

The decreased slope shown as the freezing front approaches the centre of the cylinder is due to the change in the volume of the unfrozen material with respect to the frozen/unfrozen interface. (See section 8.1.2.)

The point at which a time temperature curve (Fig. 14 to 17) departs from the temperature plateau only shows the time at which the initial freezing boundary reaches that particular location in the sample. The slope of the curve after this point is indicative of the rate at which the remaining water solidifies. Increased negative slopes indicate a rapid decrease in temperature of the frozen section, and therefore an increase in the rate at which the remaining unfrozen water is frozen. Slower freezing of the unfrozen water is shown by the reduced slopes.

The rate of the boundary advancement, and the subsequent temperature drop is directly related to the crystal structure, and therefore the fibre structure. Ice crystal nucleation was suspected to occur strictly on the outside surface. This is substantiated in the plates shown in section 8.1.3. Once the ice crystal growth has started, the heat transfer will occur more rapidly through the ice crystals, as the thermal conductivity of ice is approximately four times that of water and poultry muscle. Therefore, the water is more likely to crystallize on the ice surface, where the energy is removed more rapidly, than randomly within the protein matrix. Fast cooling rates will form smaller, more numerous ice crystals and therefore smaller fibres as the rate of energy removal is faster than the rate at which the water can diffuse through the material. As the freezing rate decreases, the diffusion rate becomes more predominant, allowing the formation of larger crystals, and thicker fibres.

The samples were all stored at -25°C after freezing. This is not likely to significantly alter the fibre structure of either the more rapidly or slowly frozen samples. Fennema (1973) found at most a 10% increase in the percentage of water frozen between -10°C and -30°C. As the protein is significantly dehydrated and compacted by -10°C, it is suspected that this structure would inhibit any further crystal growth internally. Therefore, the only reaction possible is a slight increase in the size of existing crystals, which may compact the protein fibres a little more.

8.1.2 Semi-Infinite Cylinder vs. Semi-Infinite Slab Freezing Moulds

Any factor affecting the overall rate of energy transfer from a material will influence the size and the rate of crystal growth. Therefore, the increasing insulative effect of the frozen phase between the freezant and the unfrozen phase of the precipitate, which causes a reduction in the rate of energy removal, will effect a change in the structure of the ice crystals. As the thickness of the frozen phase increases, the change in crystal structure will become greater.

The rate of heat transfer from the unfrozen phase is also affected by the geometric form in which the sample is frozen. Modifying this design so as to cause an increase or decrease in the ratio of the frozen/unfrozen interfacial area to the freezant/sample interfacial area, with respect to the distance from the freezant/sample interface, will effect a change in the rate of heat transfer and subsequently the fibre structure. The change in the fibre structure can be measured by the change in fibre density, peak force or final total solids. As will be shown in Section 8.5, a decrease in the final total solids of initially identical samples after they are frozen and heat set is indicative of an increase in the fibre density and a decrease in the peak force. Due to the nature of the product, the change in fibre structure with respect to distance from the freezant surface can best be determined by the change in the total solids. Figure 19 illustrates the change in fibre structure as shown by the change in total solids between the internal or the insulated half of the sample and the half of the sample in contact with the freezant. A ratio of greater than one, as seen in all the slab samples, shows the total solids of the samples increase with respect to distance from the freezant surface. As the samples are initially homogeneous before heat setting and exhibit no change in total solids prior to heat setting, this change in the final total solids is an indication of the degree of change of the fibre structure. It is also indicative of a decrease in the fibre density.

At all precipitate pHs and freezing rates, the semi-infinite cylinder form showed essentially no change in the total solids ratio between the insulated and contact halves, as the ratio remains close to one. However, the semi-infinite slab form shows a definite increase in the total solids between the insulated and the contact halves. The pH 4.8 precipitate shows a slightly smaller change in

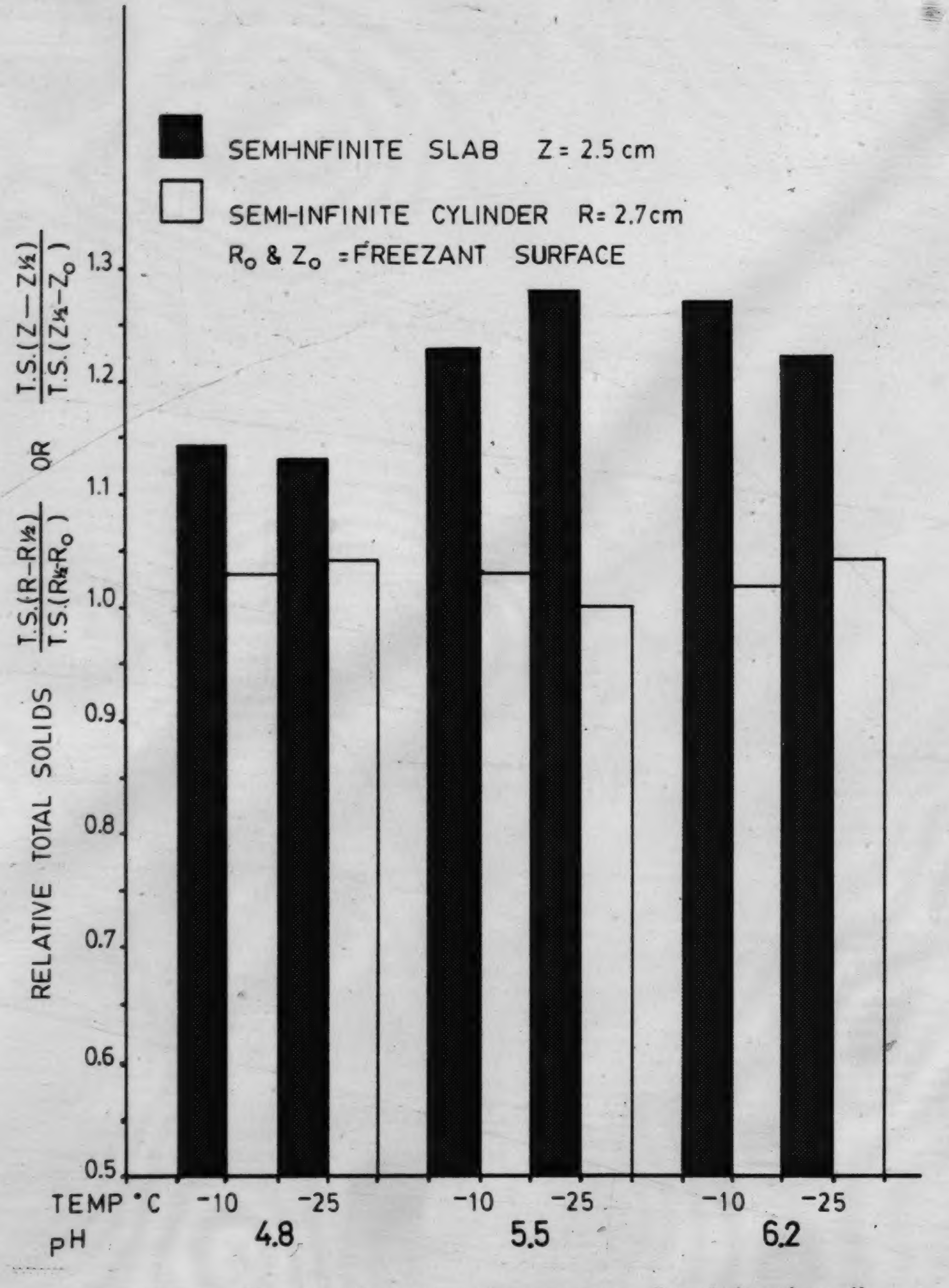

Figure 19 Effect of Freezing Temperature, Precipitation pH and Freezing Form on the Total Solids Ratio (Insulated Half/Freezant Contact Half).

total solids than the pH 5.5 and pH 6.2 precipitates.

Plate 1 illustrates the change in crystal and fibre size with respect to the distance from the freezant/sample interface with a slab form. This sample, precipitated at pH 5.5 and frozen at -25°C shows a very dramatic increase in the void area and the fibre size. Plate 2 shows a similar sample, frozen at -25°C in a cylindrical form. (The difference in colors is due to a color shifting caused by the lights). No noticeable increase in crystal and fibre size, with respect to distance from the freezant/sample interface, can be seen in the cylindrical form.

The effect of cylinder radius on the total solids ratio is shown in Figure 20. No significant difference ($p<0.05$) in the total solids ratio was found between the 1.2, 2.0, and 2.7 cm radius cylinders. However, the total solids ratio of the 4.5 cm radius cylinder and the 2.5 cm thick slab were above one and were significantly different ($p<0.05$) from each other and the other three cylinders. As expected, the semi-infinite slab showed the greatest total solids ratio, although the 4.5 cm cylinder was approaching this.

The time/temperature/distance profiles, (Figs. 14 to 17) for a 2.7 cm radius cylinder show an increase in the rate of the freezing boundary advancement and the rate of temperature drop, nearer the centre of the cylinders. This agrees with the results found by Meryman (1966). The change in the rate of the frozen boundary advancement is due to the change in the overall heat transfer coefficient and the

Freezant Surface
Insulated Surface

Plate 1 Effect of Freezing Form on Fibre Structure. Protein Concentrate at pH 5.5, Frozen at -25°C in a 2.5 cm deep Semi-Infinite Slab. (28:1 Magn.)

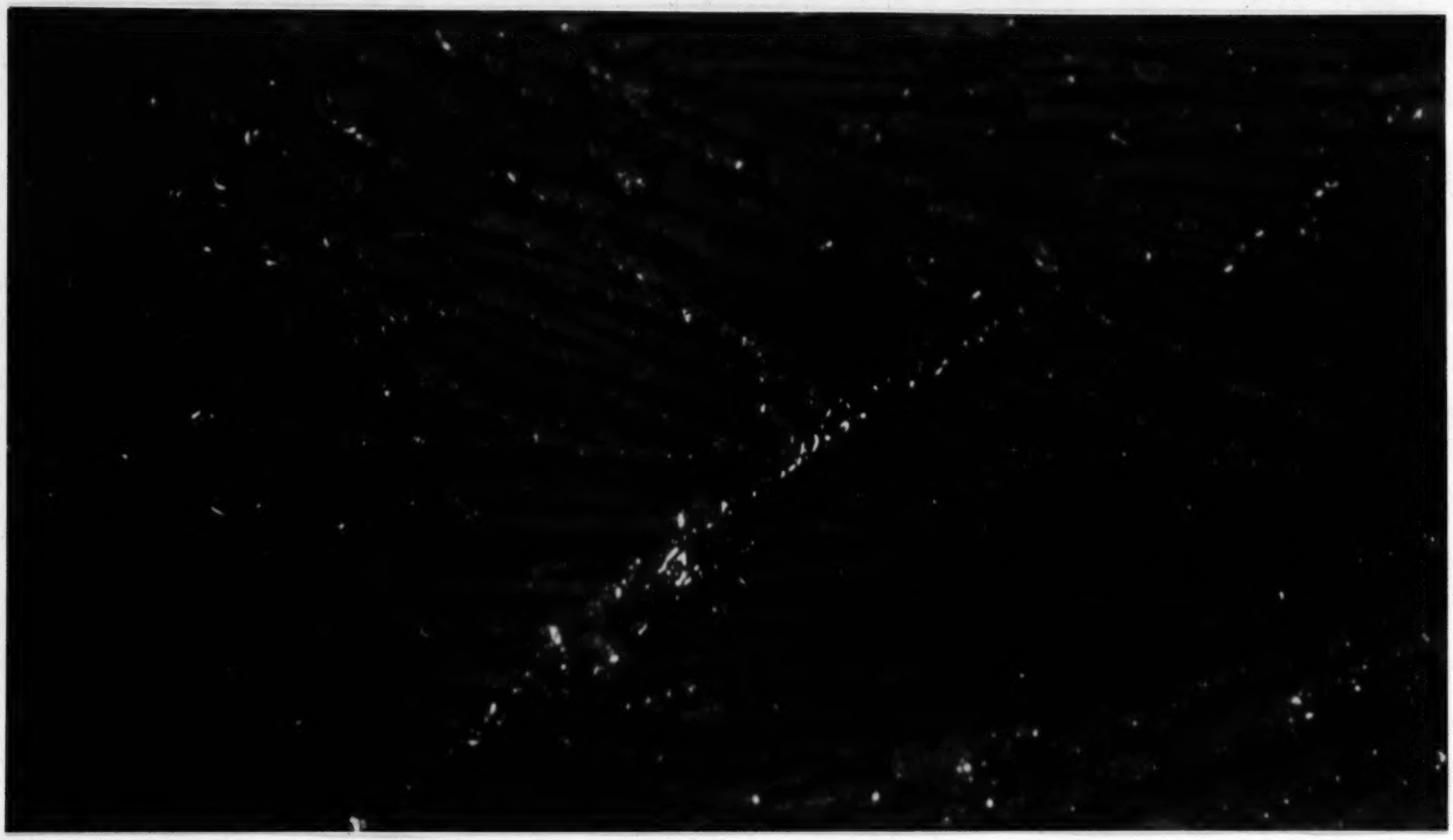
Freezant Surface
Center

Plate 2 Effect of Freezing Form on Fibre Structure. Protein Concentrate at pH 5.5, Frozen at -25°C in a 2.7 cm Radius Semi-Infinite Cylinder. (28:1 Magn.)

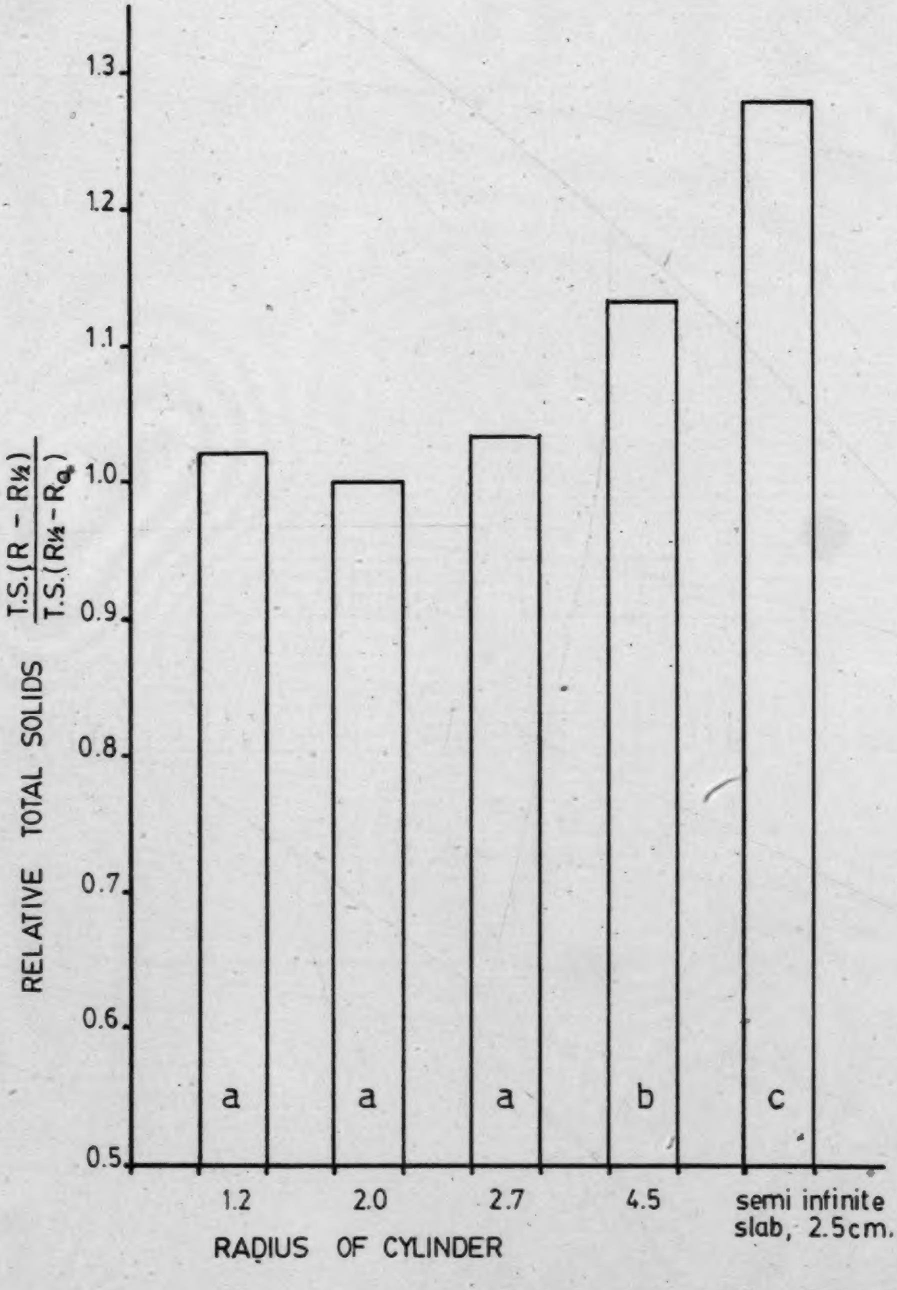

Figure 20 Effect of Cylinder Radius on the Ratio of Total Solids of pH 5.5 Precipitate Frozen at -25°C. a,b,c, significant difference ($p \leqslant 0.05$)

change in the ratio between the unfrozen/frozen interfacial area and the unfrozen volume. The overall heat transfer coefficient is affected by the change in thickness of the frozen zone and the change in the area of the unfrozen/frozen interface.

In a semi-infinite slab, the equation for the overall heat transfer coefficient is

$$\frac{1}{UAo} = \frac{1}{hoAo} + \frac{\Delta X}{kAo} + \frac{1}{hiAo}$$

where: U = overall heat transfer coefficient
Ao = surface area
ho = freezant/sample surface heat transfer coefficient
hi = unfrozen/frozen surface heat transfer coefficient
k = thermal conductivity of frozen phase
ΔX = distance of frozen boundary from freezant.

As the area remains essentially constant with respect to X, the only increase in the resistance to heat transfer is the increased thickness of the frozen phase. At some distance X and any point beyond that distance, the rate of heat transfer through the frozen phase will be the limiting factor. Therefore, the crystal growth, with respect to an increasing frozen phase thickness, will simulate that found with an higher ambient temperature. The crystals will increase in size and decrease in numbers, as seen in Plate 1, producing fewer, larger fibres.

A semi-infinite cylindrical form is substantially different. Only the freezant/sample interface resistance is independent of the thickness of the frozen phase. The previous equation for the overall heat transfer coefficient becomes,

$$\frac{1}{UA} = \frac{1}{hoAo} + \frac{\Delta r}{kAlm} + \frac{1}{hiA1}$$

where: Δr = radial distance of frozen boundary from freezant
Ao = freezant/sample interface area
Alm = log mean area
A1 = unfrozen/frozen interface area.

The resistance of the frozen phase is a linear function of the log mean area. At the same time, the resistance at the unfrozen/frozen interface increases due to the decreased area. Therefore as the distance increases from the freezant surface, the overall resistance to heat flow increases more rapidly than with a semi-infinite slab. As the frozen phase advances, the volume of the unfrozen material decreases with respect to the surface area, at a ratio of r/2, causing a reduction in the total latent heat of fusion released for a given distance Δr. At some critical thickness of the frozen phase, the decrease in unfrozen volume will have a greater positive effect towards increasing the frozen boundary advancement than the negative effect of the increase in the overall resistance. Fig. 18 shows the increase in the rate of the frozen boundary advancement with 2.7 cm radius cylinders as the front approaches the centre.

Increasing the radius of the cylinder (Fig. 20) produced samples which showed a definite increase in the total solids ratio, and a slight increase in the ice crystal size was noticed. This would suggest that a critical cylinder radius exists, after which point, the cylinder would simulate the results found using a semi-infinite slab.

From a theoretical side, the cylindrical form should limit the size of non-normal crystals, more than the slab form. Fig. 11 (de Quervain, 1975) shows that the normal crystals should overtake the parallel crystals. The cylindrical form should therefore enhance this due to the convergence of the normal crystals. However, initially the cylindrical form favors the formation of non-normal crystals, especially at slow freezing rates, due to the curvature of the surface. Although it was virtually impossible to measure this, the cylinders did have a greater tendency to form non-normal crystals at the surface than the slabs, though these were rapidly overtaken by the normal crystals at the faster freezing rates. The slower freezing rates did not control the non-normal crystals, nor overtake them as rapidly. This is illustrated by Plates 10 and 6 in Section 8.1.3.

The cylindrical forms therefore allow for the production of larger samples with consistent fibre size than the slab form. Opposing freezant surfaces could be used to increase the thickness of the slabs, without a fibre size change, though this could create a central plane where there may be limited intermeshing of the fibres. The cylindrical form, using sausage casings, may also be more appropriate for usage in the industry. The major advantage of the slab design is the formation of parallel fibres rather than radial fibres, though this advantage is dependent on the products' intended use.

8.1.3 Fibre Structure Characteristics

The fibre structure characteristics were found to be dependent on both the rate of heat removal and the nature of the proteinaceous material. Although the heat transfer dictated the gross fibre structure by controlling the ice crystal formation, the protein material characteristics had a very strong influence of the fibre structure characteristics. To a certain extent, these characteristics can be measured objectively, as shown in the following sections. However, many of the effects of these parameters are difficult to quantify and therefore require a visual record.

Altering the rate of energy removal, (ambient temperature -5, -12, -22, and -32°C) and the pH of the protein material (pH 4.5, 5.0, 5.5, and 6.0) changed the fibre structure as shown in Plate 3. This picture has a magnification of 0.84:1.0. Although the shrinkage of the cylinders due to heat setting was not measured during the experimentation, the photographs show a 15 to 25% reduction in size. The greatest shrinkage appeared with the low pH, slow freezing samples while the high pH, fast freezing exhibited the least shrinkage. The degree of shrinkage is attributed to the size of the ice crystals formed, the degree of crosslinking (explained in greater detail in Section 8.2) and the water holding capacity of the product.

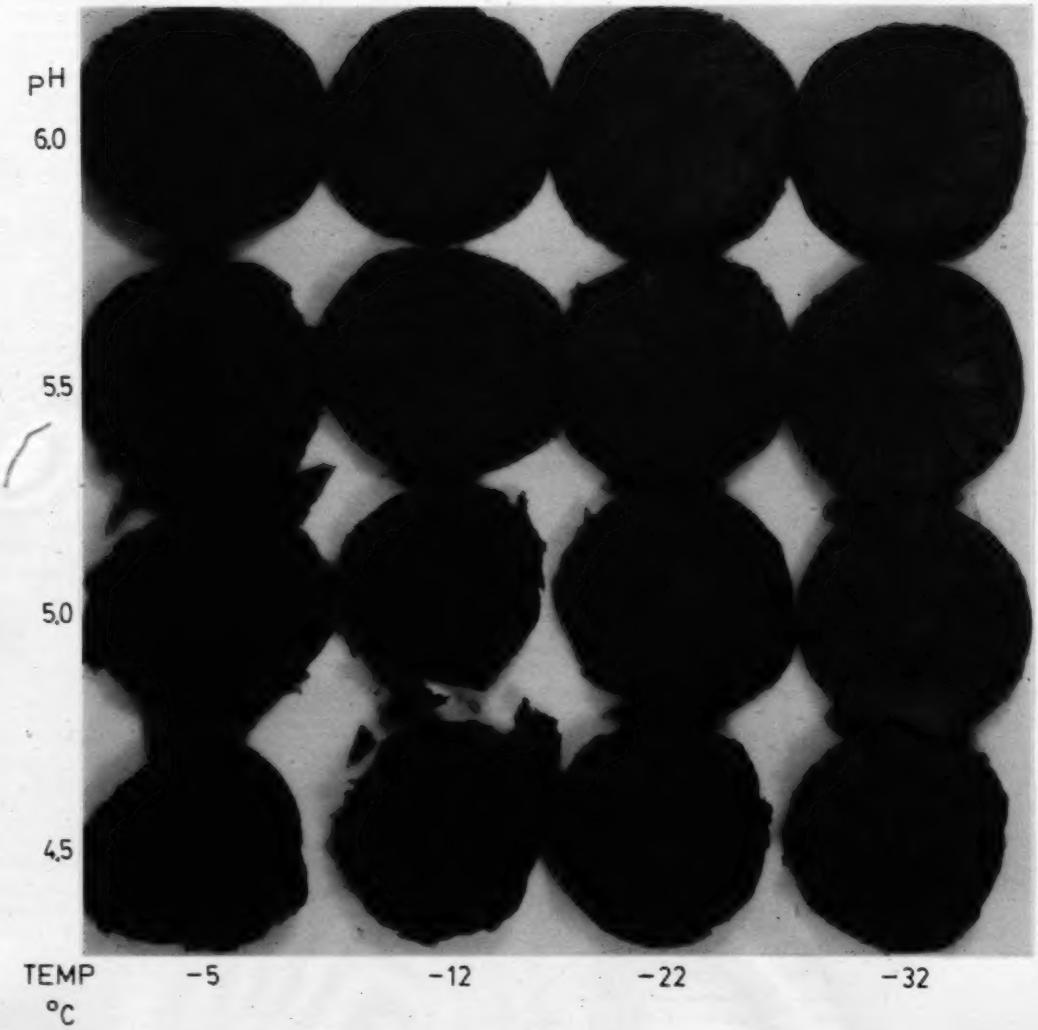
pH
6.0
5.5
5.0
4.5
TEMP °C
−5
−12
−22
−32

Plate 3 Effect of Freezing Temperature and pH of the Protein Concentrate on the Structure of Freeze-Texturized Protein.

A greater collapse of the structure, and loss of water will occur with slowly frozen, low pH samples, where large voids are present. Smaller crystals with greater crosslinking and a greater water holding capacity of the protein allow the fibre structure to retain its shape upon heating.

Although the photograph is somewhat dark, the color trend, showing a pinkish color at pH 6.0 to a brown-pink color at pH 4.5 can be clearly seen. Changes in the fibre structure are also clearly visible. Large, relatively randomly oriented voids can be seen in the slow freeze, low pH products. The size of the voids decreased while the general cohesiveness of the product increased with increases in the pH and the rate of freezing. Increasing the magnification of the picture shows fibre structure in greater detail. The magnified photographs show samples with the greatest change in structures.

Plate 4 shows a pH 4.5 sample frozen at -5°C. The fibres (or more specifically fibre planes) are thick, easily identifiable and relatively randomly oriented. Large voids are apparent with little or no visible crosslinking. This allows easy flaking of the fibres, reducing the cohesiveness of the sample. The fibres parallel to the surface (upper right corner) penetrate into the sample, and are only slowly inhibited by fibres normal to the surface. The large, randomly oriented fibres are typical of this freezing temperature, though there is some decrease with increased pH. The lack of fibre crosslinking is typical of the pH 6.5

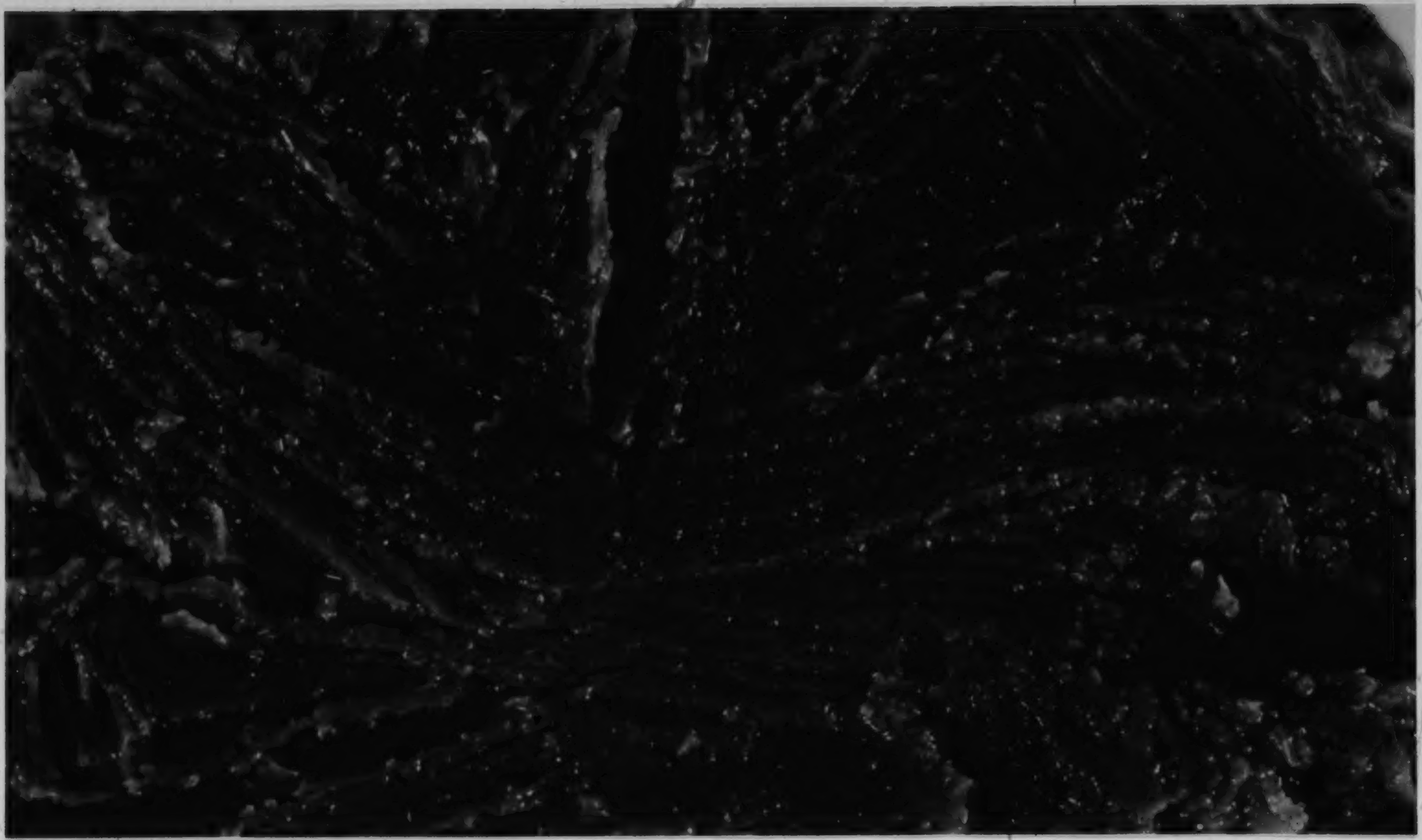

Plate 4 Protein Concentrate at pH 4.5, Frozen at -5°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(7:1 Magn.)

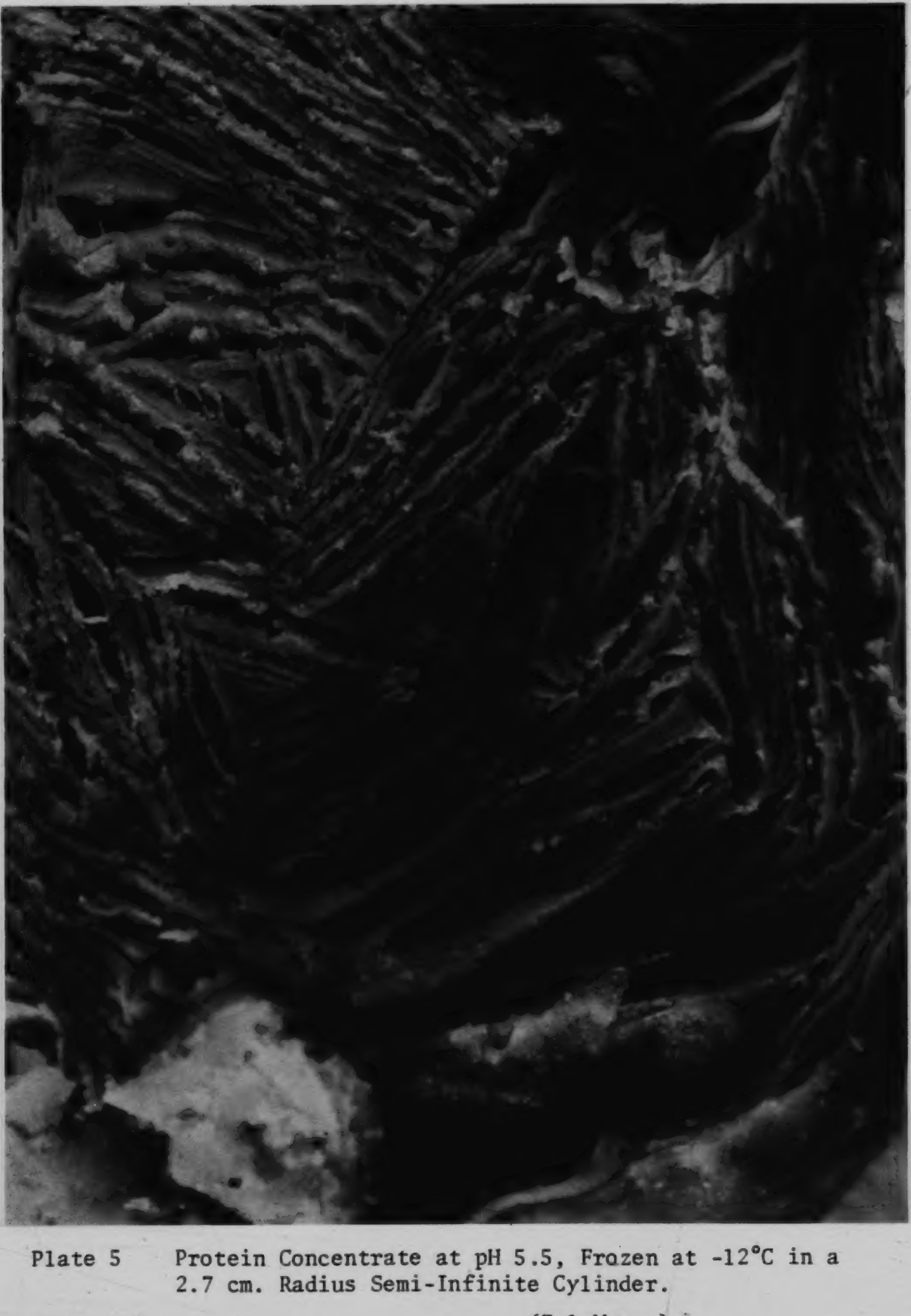

Plate 5 Protein Concentrate at pH 5.5, Frozen at -12°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(7:1 Magn.)

Plate 6 Protein Concentrate at pH 6.0, Frozen at -12°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(7:1 Magn.)

samples.

Increasing the freezing rate by decreasing the ambient temperature to -12°C, and increasing the pH to 5.5 (Plate 5) and 6.0 (Plate 6) shows a dramatic decrease in the fibre size and an increase in the fibre density. The pH 6.0 sample shows regions with slightly higher fibre densities than the pH 5.5 sample. The fibre orientation is similar to that seen in Plate 4, with a number of fibres non-normal to the surface. The pH 6.0 sample (Plate 6) shows a high degree of fibre crosslinking, which is limited in the pH 5.5 sample. This extensive crosslinking is found in all the pH 6.0 samples. Plate 7 shows a three dimensional picture of a pH 5.0 sample, frozen at -12°C and magnified 28 to 1.

Decreasing the ambient temperature to -22°C, (Plate 8) increases the fibre orientation significantly so that the majority of the fibres appear to radiate from the centre. The growth of fibres which are non-normal to the surface appear to be controlled or overtaken by the normal fibres, or more specifically the normal ice crystals as shown in Fig.11, (de Quervain, 1976). The fibre density is increased further, while the fibre thickness is decreased. Areas of parallel fibre structure appear similar to extruded protein products.

Freezing at -32°C (pH 5.0, Plate 9, pH 5.5, Plate 10, pH 6.0, Plate 11) increased the degree of orientation and the fibre density further. However, there are still some fibres which are not normal to the freezant/protein

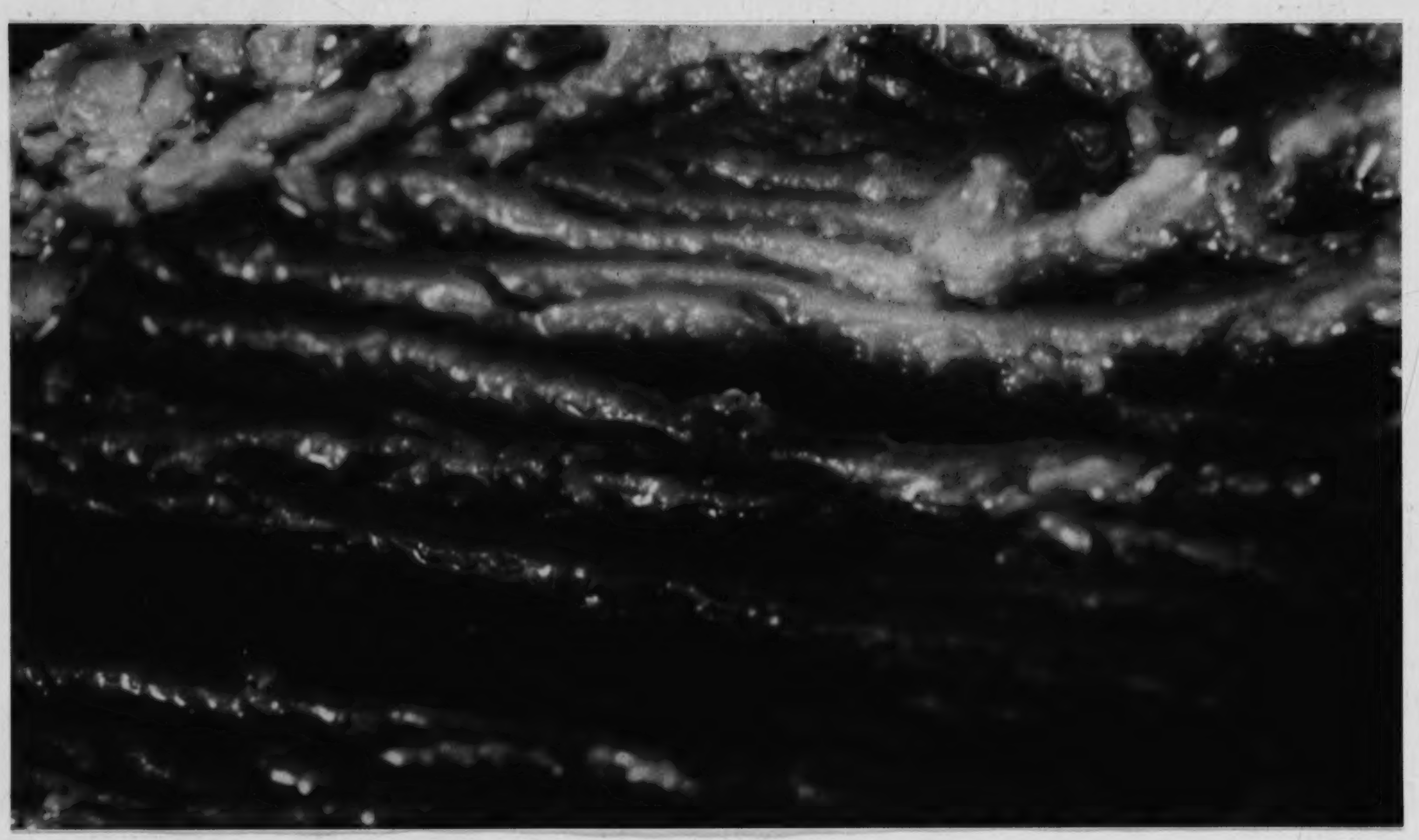

Plate 7 Protein Concentrate at pH 5.0, Frozen at -12°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(28:1 Magn.)

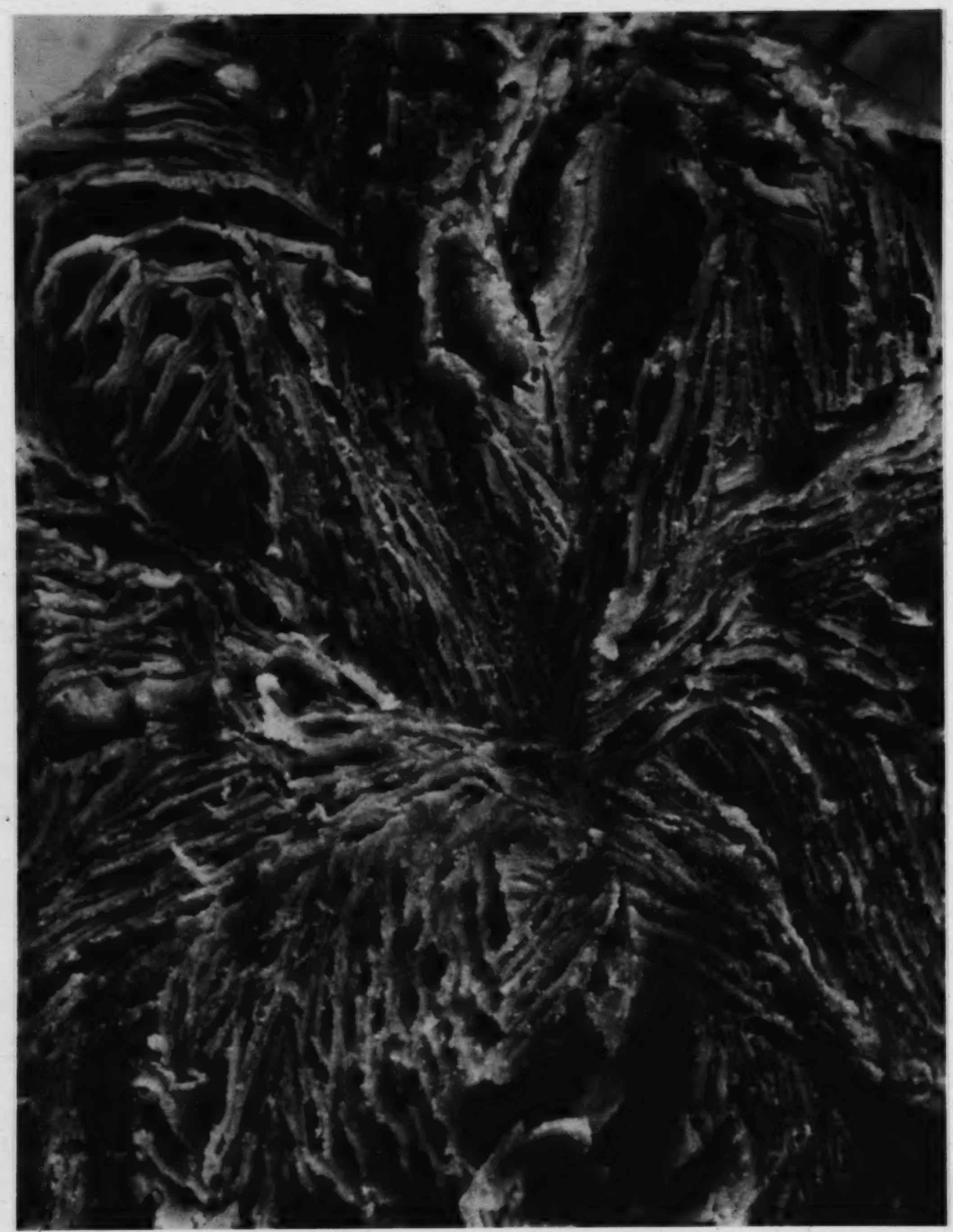

Plate 8 Protein Concentrate at pH 5.5, Frozen at -22°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(7:1 Magn.)

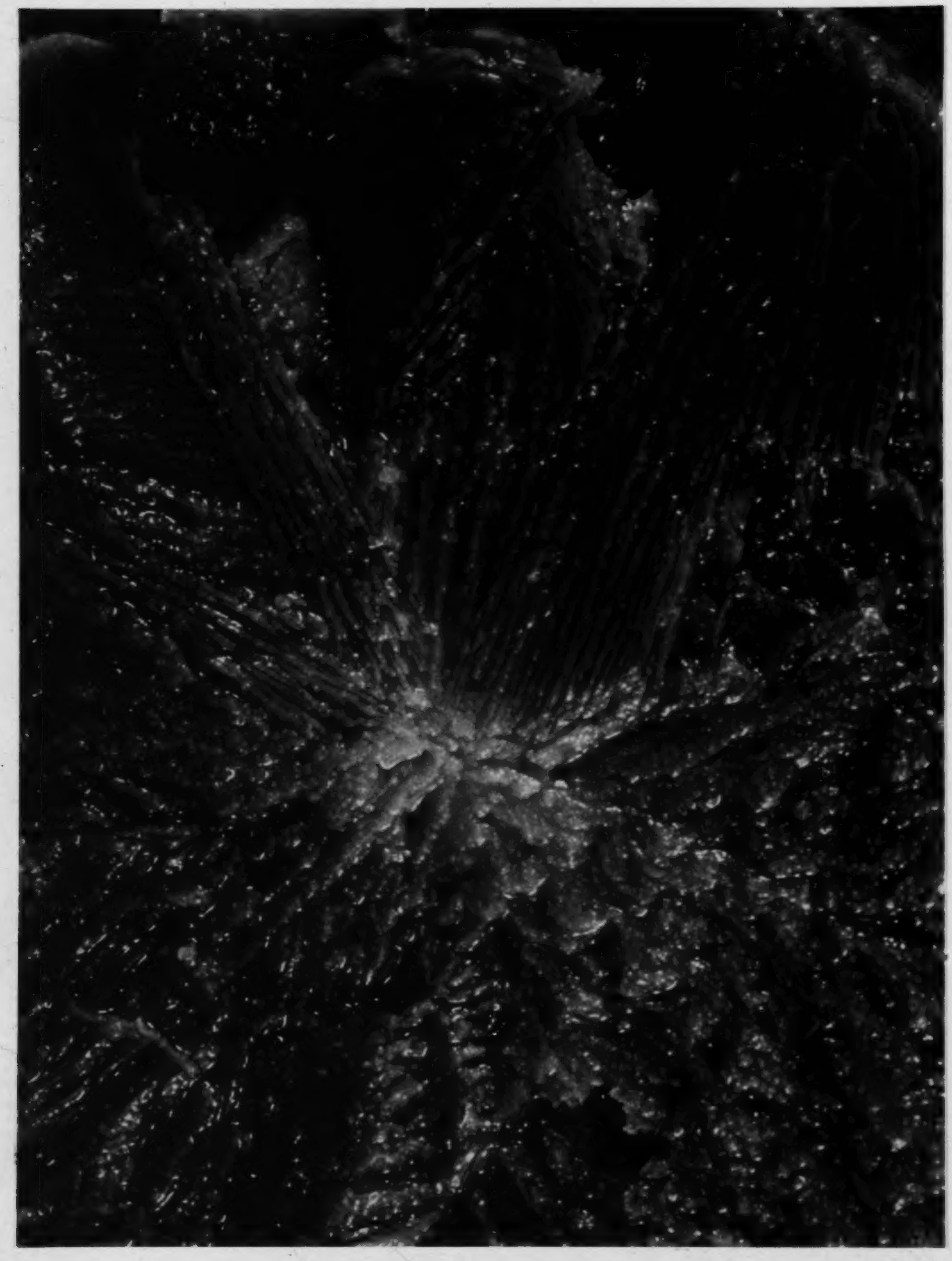

Plate 9 Protein Concentrate at pH 5.0, Frozen at -32°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(7:1 Magn.)

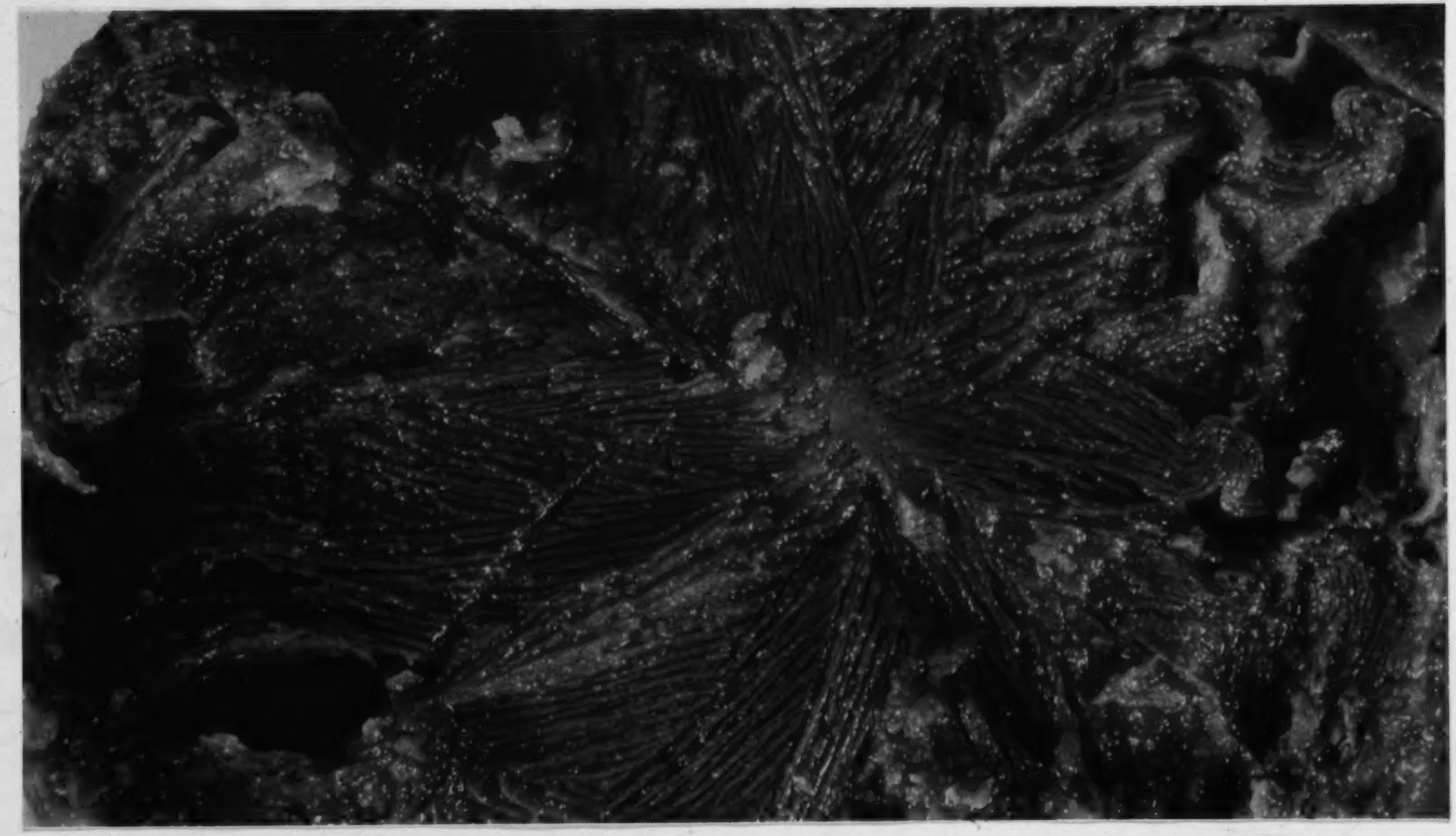

Plate 10 Protein Concentrate at pH 5.5, Frozen at -32°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(7:1 Magn.)

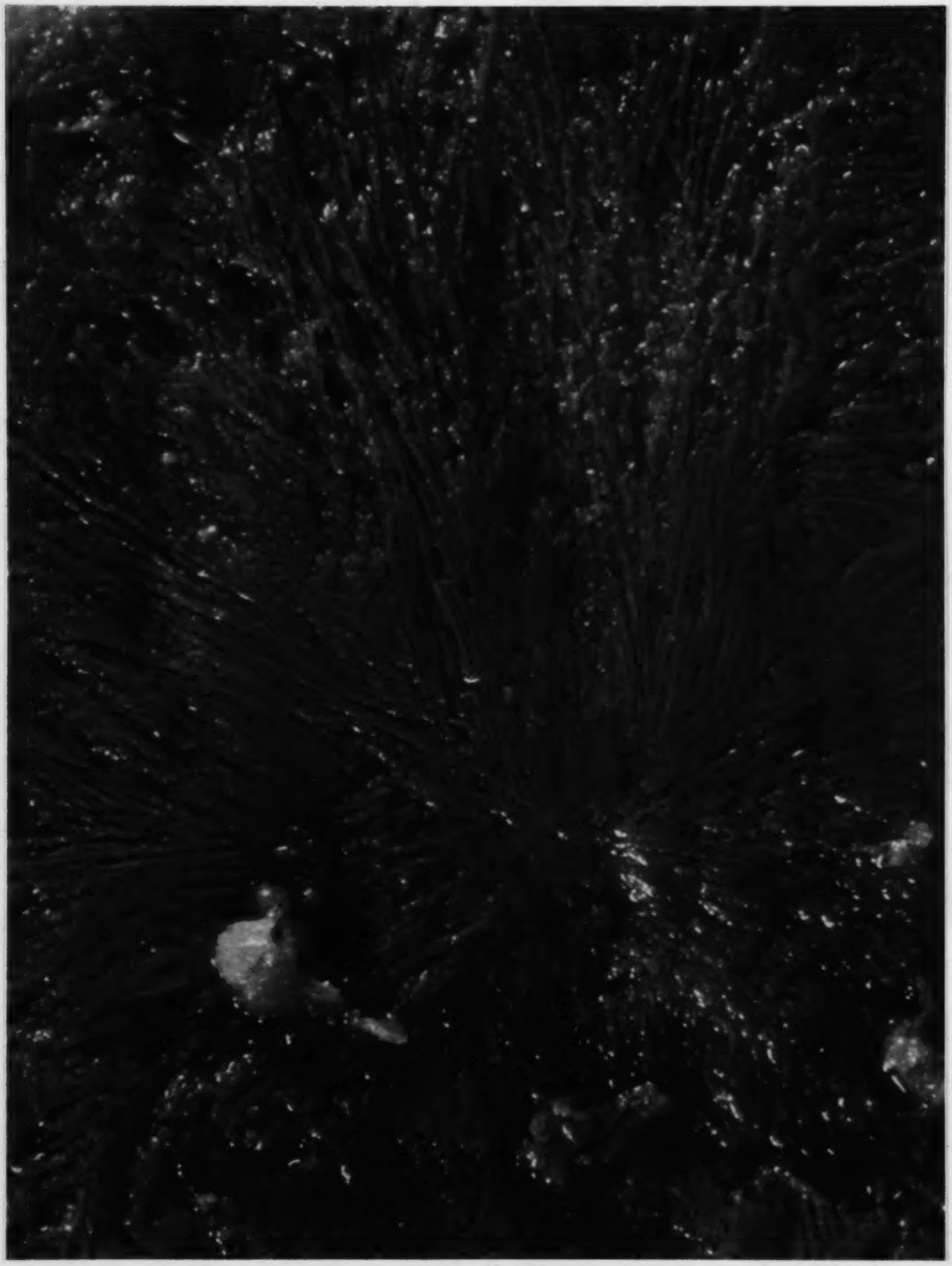

Plate 11 Protein Concentrate at pH 6.0, Frozen at -32°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(7:1 Magn.)

interface. The fast freezing rates cause the normal crystals to predominate and overtake the non-normal crystals very rapidly. The fibres in the pH 6.0 sample (Plate 11) are less discernable than the fibres in the other two samples. This may be due to a greater degree of crosslinking between the fibres, which would tend to mask the individual fibres at this magnification. Plate 10 illustrates the effect that entrapped air bubbles can have on the structure. It can cause collapse of the fibre structure, as seen in the top right corner, or just discontinuities in the fibres as seen in the bottom left corner. Plate 12 shows the pH 5.0 samples at a 28:1 magnification, where some of the fibre planes are at an angle to the photographic plane (ie. exhibiting the sides of the fibres). Very limited fibre crosslinking is visible.

Increasing the total solids content of the precipitates at a constant pH prior to freezing can inhibit large crystal growth, as it limits the amount of available water for freezing. This increases the resistance of water transfer through the material to the crystal surface along with the solute and solids movement away from the crystal surface. Plates 13 and 14 show the difference in fibre structure with pH 5.5 samples with initial total solids of 15.9% and 19.3%, frozen at -25°C. The void sizes are noticeably larger and appear to be more numerous with the 15.9% T.S. sample. Although the void sizes are smaller with the 19.3% T.S. sample, the fibres appear somewhat larger and are not as

Plate 12 Protein Concentrate at pH 5.0 Frozen at -32°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(28:1 Magn.)

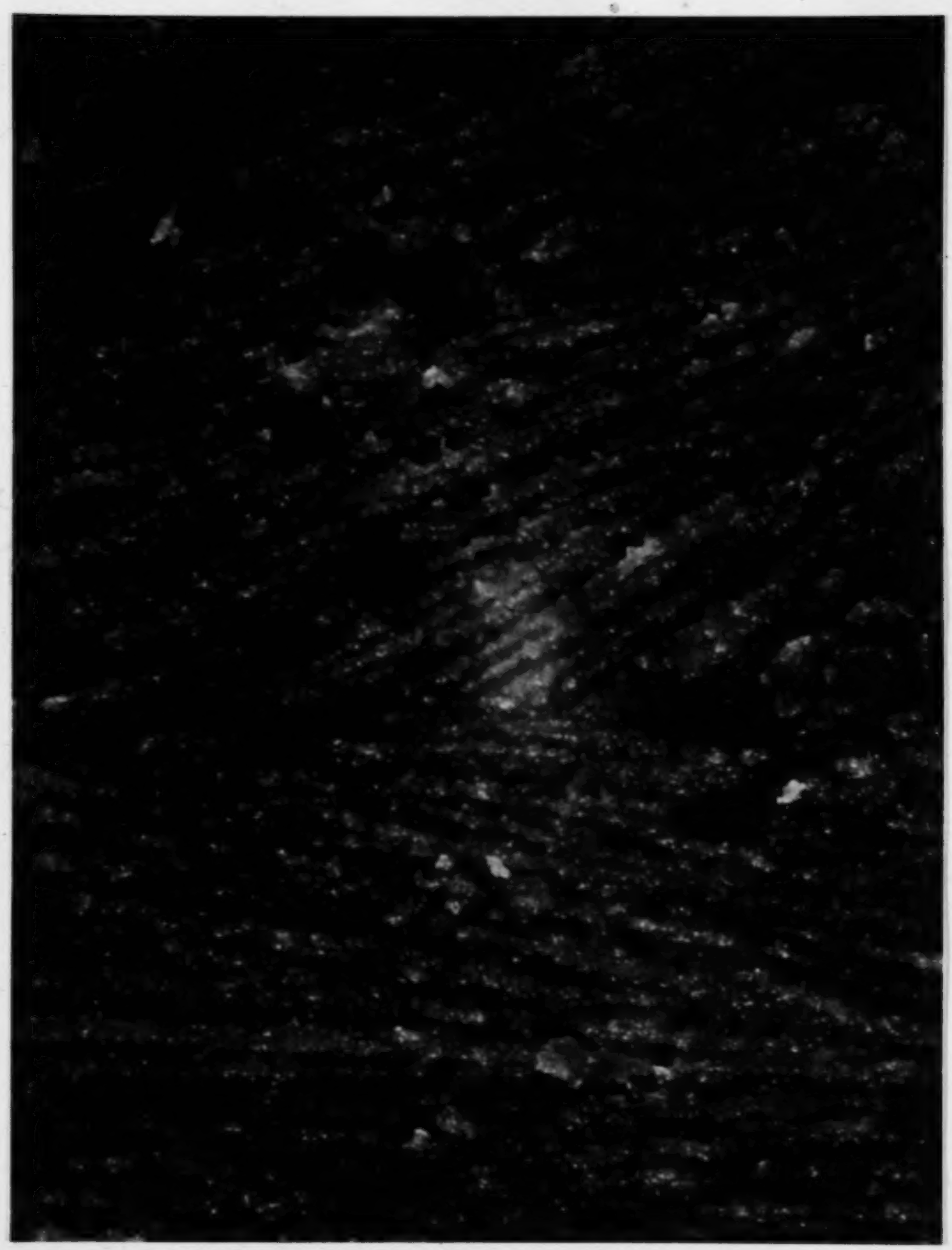

Plate 13 Effects of Total Solids on Fibre Structure: Protein Concentrate at pH 5.5, 15.9% Total Solids, Frozen at -25°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(28:1 Magn.)

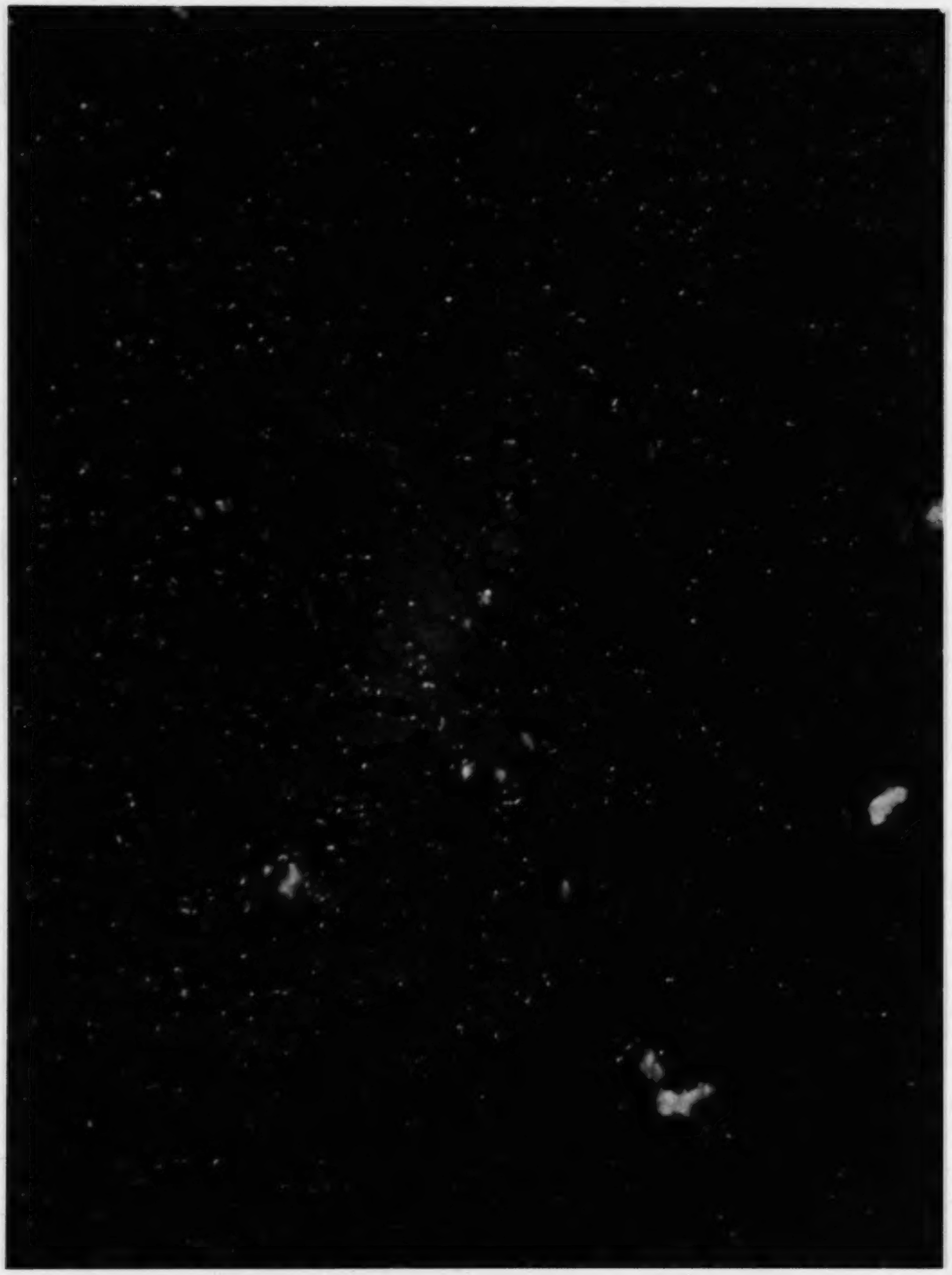

Plate 14 Effects of Total Solids on Fibre Structure. Protein Concentrate at pH 5.5, 19.3% Total Solids, Frozen at -25°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(28:1 Magn.)

physically independent of each other. Air bubbles can be seen in Plate 13, disrupting the fibre structure.

Plate 15 shows a picture of the textured protein when the protein was frozen in a finite slab, with all sides in contact with the freezant surface. The pH 5.5 protein was frozen at -20°C in a 1.5 cm deep, 5.5 cm diameter dish, with no insulated surface. The fibres are generally randomly oriented, with limited parallel fibres occurring. The sample does not exhibit a consistent fibre structure. (The yellow tint was caused by a lighting induced color shift).

Although not specifically investigated in this work, a Kori-tofu like structure, though considerably denser, was produced with this protein source. A very low total solids (6%), pH 5.0 sample was frozen at -10°C in a natural convection freezer in the aforementioned 5.5 cm diameter dish. Of concern in the picture of this product (Plate 16) are the small protein sacs. They were caused by rapid addition of a high strength acid to a poorly mixed protein extract, causing the formation of pockets of high acidity. It is important that these pockets do not occur as they can disrupt the fibre structure.

Thorough discussion of possible biochemical reactions which may occur during the freezing process would be speculative from these results and is beyond the scope of this work. However, several mechanisms take place which may affect the protein fibre structure.

It is unlikely that any of the protein denaturation

Plate 15 Non-Directional Freezing. Protein Concentrate at pH 5.5, Frozen at -20°C in a Non-Insulated Slab Form.

(28:1 Magn.)

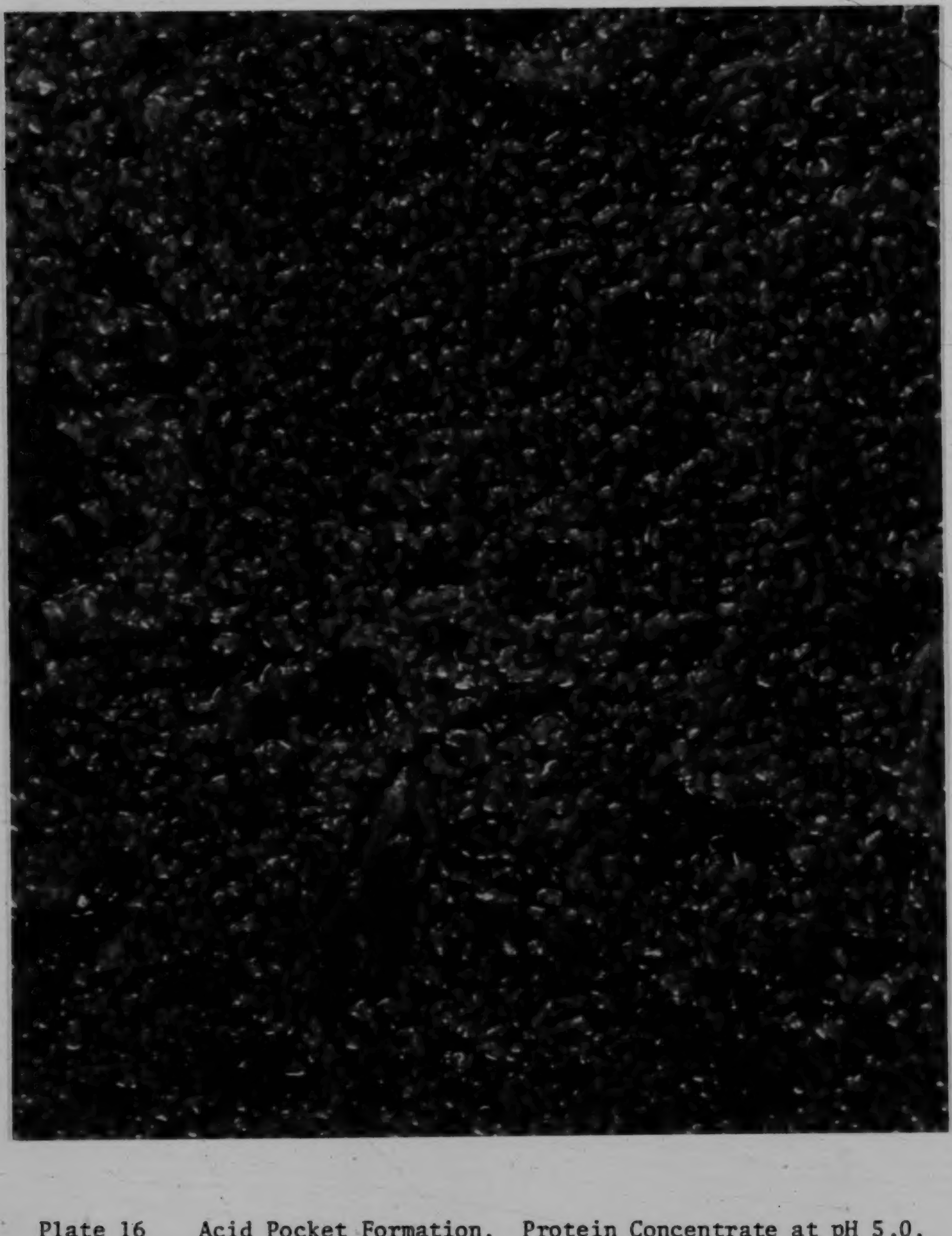

Plate 16 Acid Pocket Formation. Protein Concentrate at pH 5.0, 6% Total Solids, Frozen at -10°C in Non-Insulated Slab Form Showing Presence of Acid Formed Protein Pockets.

(28:1 Magn.)

found by Finn (1932) to occur over an extended storage period, and at relatively high sub-zero temperatures (-3 to -5°C), will take place in the process investigated here. Finn (1932) found that the amount of protein denaturation dropped rapidly after -5°C and was vastly reduced with storage periods shorter than thirty days (Fig. 13). The production of kori-tofu requires similar conditions (Wabanabe _et_ _al_., 1974). However, it was found in this work that freezing at this high an ambient sub-zero temperature produced fibres which were too large, unconnected and unoriented, and therefore not suitable for texturizing this protein source. Storage for extended periods (>2 days) at these temperatures, after freezing at lower temperatures was not investigated. It is suggested that such a storage would lead to some degree of recrystallization, causing a greater degree of separation between the fibres and breaking some of the crosslinks. It also appears not to be required as intact fibres were formed without this temperature or storage period.

Protein/protein interactions are still likely to occur due to the concentration of the material as the water freezes out. A greater effect perhaps, due to the short storage periods, is the compaction of the concentrated protein material due to the increased volume occupied by the ice crystals. The frozen water increases in volume by approximately 9%. Some of this resulting increased pressure may be absorbed by an increase in the size of the total sample. To some extent, however, the concentrated proteinaceous material must decrease in volume due to this increased pressure.

At the same time as the protein is concentrated, other solutes will also increase in concentration. By -20°C, researchers have found that in most foodstuffs, approximately 90% of the water is frozen out, leaving a higher concentration of the solutes in the protein material. The alkaline extraction, followed by acid precipitation with HCl produces an NaCl solute in the precipitate, with an initial concentration of 0.04 to 0.07 moles/kg precipitate. Assuming initial total solids of approximately 12%, freezing the sample can increase the concentration to between 0.19 to 0.34 moles NaCl/kg precipitate or 0.45 to 0.85 molar solutions (without the protein). This is well within the range that Young (1975) used to extract proteins from mechanically deboned meat. This would therefore suggest that some solubilization or unfolding of the proteins could occur, dependent on the interrelating effects of decreased temperature (ie. reduced reaction rates) and increased pressure. If some unfolding does occur, the pressure from the compaction may align the proteins such that they occupy a minimal total volume, increasing the possibility of protein/protein interactions.

8.2 Fibre Crosslinking

A major drawback of some of the alternate methods of protein texturization, especially spun-fibre, is the necessity of the addition of binding agents such as egg albumin, to bind the fibres together. This is unnecessary in freeze-fibre formation, as the fibres are joined at the ice crystal boundaries, and to some extent through the voids in the crystal structure where crosslinking or bridging has occurred.

The formation of crosslinks between the fibres appears dependent primarily on the pH of the precipitate and the freezing rate. The crosslinks are formed in the interstitial areas between the branches of ice crystals, running perpendicular to the large fibres. Figure 21 shows a schematic of an ice crystal with the interstitial areas where the concentration of the proteinaceous material occurs. The crosslink formation may be due to the concentration effect on possible reactions between the proteins, caused by the water extraction, and the compaction effect, caused by the increase in volume upon crystallization of ice.

Crosslinking appears to occur predominantly with the more rapidly frozen, high pH precipitates. Plate 17 shows a number of small crosslinks in a pH 6.0 precipitate frozen at -32°C. A reduction from pH 6.0 to pH 5.0, with the same freezing rate, reduced the extent of crosslinking (Plate 18). Decreasing the pH to 4.5, combined with increase in the

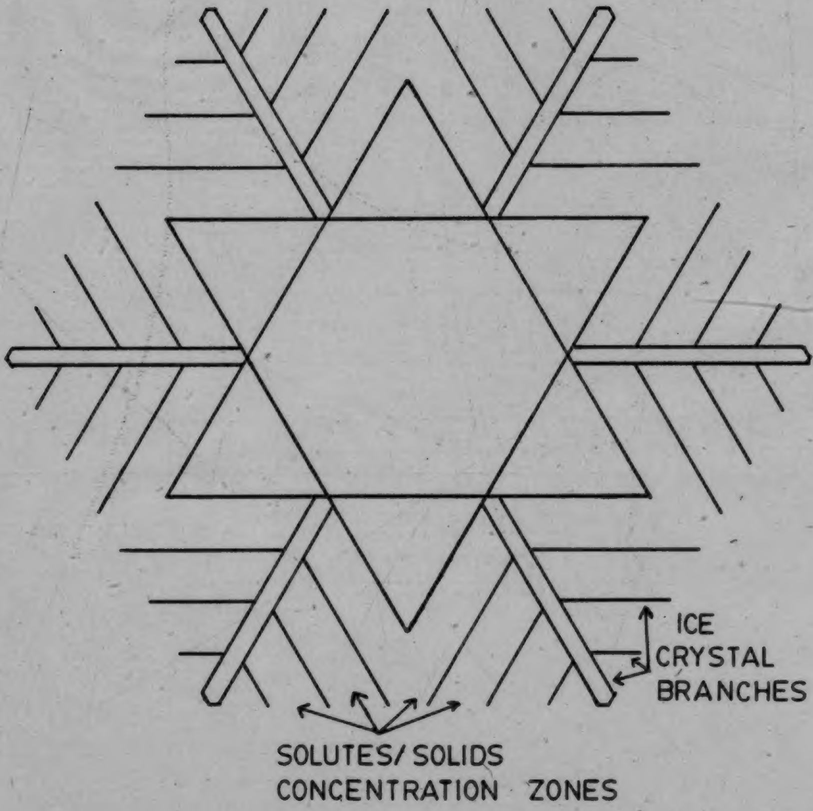
ICE
CRYSTAL
BRANCHES
SOLUTES/SOLIDS
CONCENTRATION ZONES

Figure 21 Schematic of Hexagonal Crystal Growth.
(from de Quervain, 1975)

ambient temperature to -5°C, caused a vast reduction in the number of crosslinks, (Plate 19).

During the acidification step to precipitate the proteins it was noted that at pH 4.5 and pH 5.0, the protein formed small, though visible floccules. At pH 6.0, the protein appeared to be suspended homogeneously within the solution, with a greatly reduced degree of flocculation occurring. After the solution was allowed to set for several hours, the protein in the pH 6.0 solution remained suspended, while it settled at pH 4.5. The degree of settling and flocculation decreased with increasing pH, generally following the water-holding capacity vs. pH curve for poultry meat (Fig. 6).

The freezing process causes a concentration and compaction of the proteins. It would appear that the floccules formed at lower pHs are less capable of being held together upon freeze concentration and subsequent heat setting, than the more dissolved proteins at pH 6.0. This may be due to the non-homogeneous nature of the precipitate in the crosslinks (i.e. areas of high and low total solids) or the more folded state of the proteins at the low pHs. The more unfolded structure of the proteins at pH 6.0 may allow for greater protein/protein interactions and bonding, than the lower pH 4.5 samples.

As shown in Plate 17, the rapid freezing produces relatively thin and numerous fibres, separated by small voids. Rapid freezing also causes a greater degree of

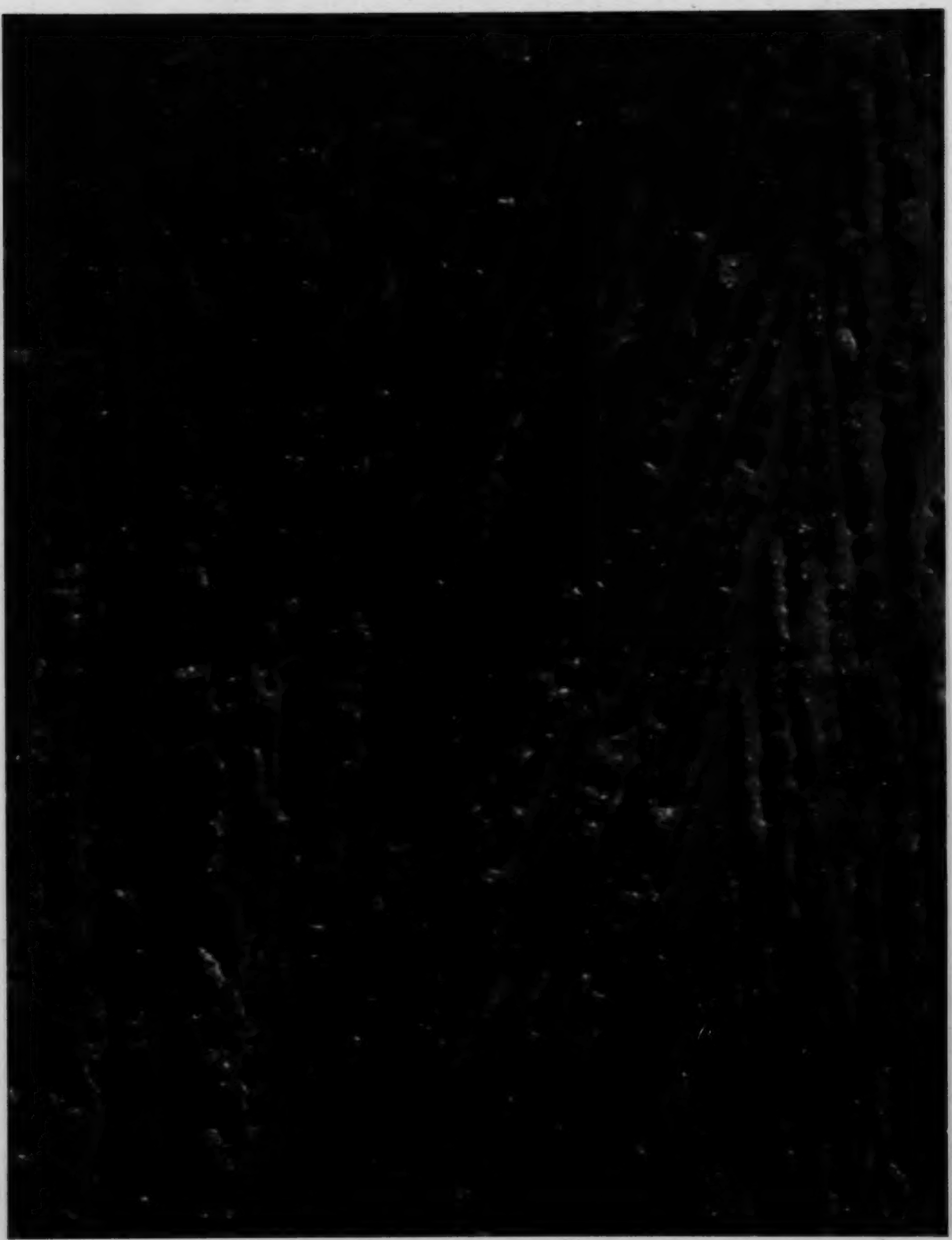

Plate 17 Effect of Freezing Temperature and Protein pH on Crosslink Formation. Protein Concentrate at pH 6.0, Frozen at -32°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(28:1 Magn.)

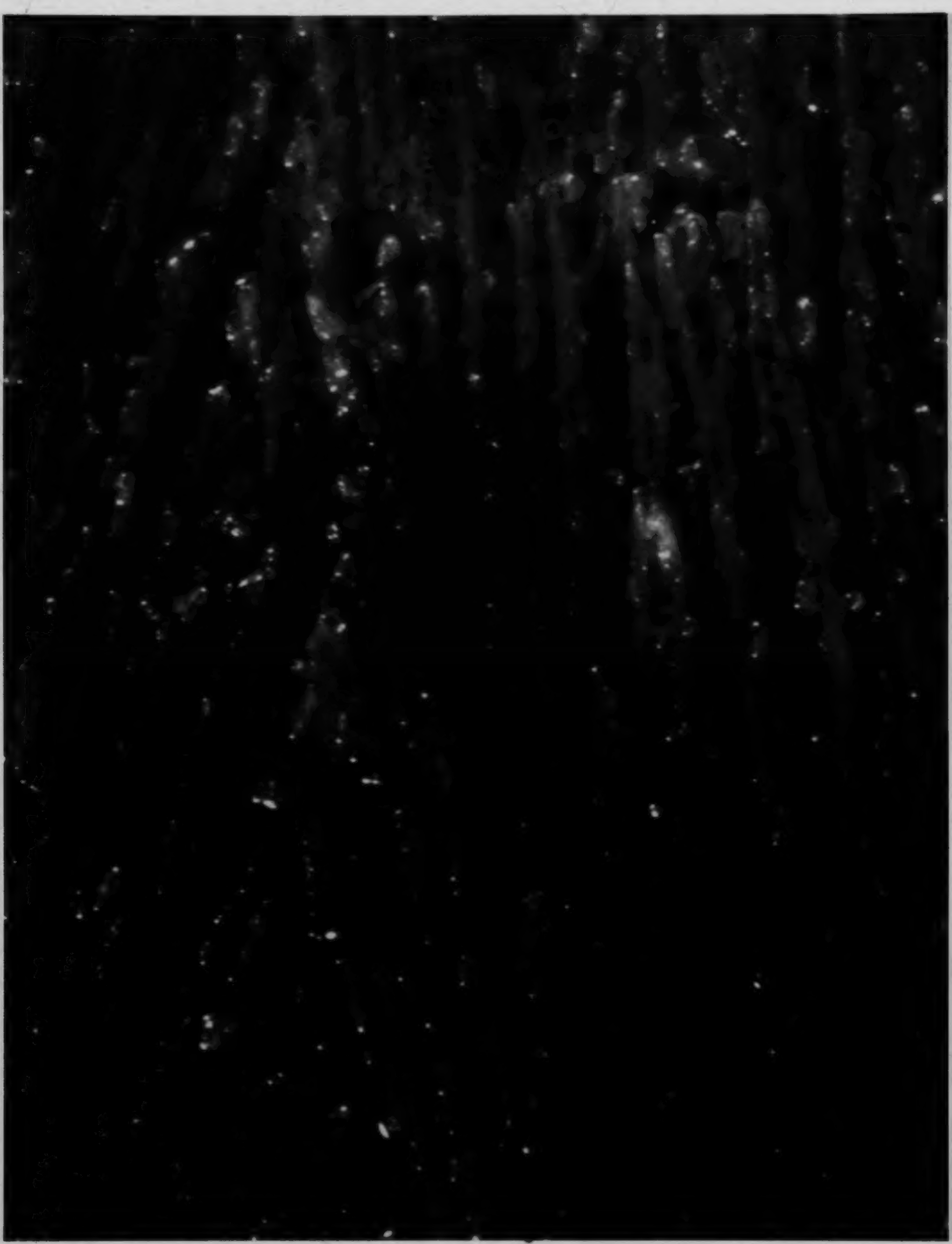

Plate 18 Effect of Freezing Temperature and Protein pH on Crosslink Formation, Protein Concentration at pH 5.0, Frozen at -32°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(28:1 Magn.)

Plate 19 Effect of Freezing Temperature and Protein pH on Crosslink Formation, Protein Concentration at pH 4.5, Frozen at -5°C in a 2.7 cm. Radius Semi-Infinite Cylinder.

(28:1 Magn.)

crystal branching, thereby increasing the number of sites where the crosslinks form. Inversely, slower freezing creates larger voids between the fibres, and a lesser degree of crystal branching. Therefore, fewer, longer, and less stable crosslinks are formed, which are susceptible to breakage or damage due to any external force applied to the product, thereby reducing the interfibre binding. The increased degree of crystal branching at more rapid rates would have a much greater binding effect between the fibres.

8.3 Fibre Density

Fig. 22 shows the effects of the freezing temperature and the protein pH on the fibre density, (number of fibres per unit length). These values are partially subjective as sections were chosen which did not contain major flaws.

The fibre density was found to increase while the fibre size or thickness decreased with increased freezing rates. This agrees with the curves shown by Hallett (1968, Fig. 10) describing the nucleation and crystal growth rate. The fibre density increases most rapidly with respect to freezing temperature to -22°C, after which changes in temperature do not have as great an effect. The freezing boundary advancement curves (Fig. 18) show a similar relationship. Therefore, changes in temperature at low freezing temperatures will have a smaller effect on the fibre density than at higher subzero temperatures. Temperature fluctuations at lower temperatures will be less detrimental

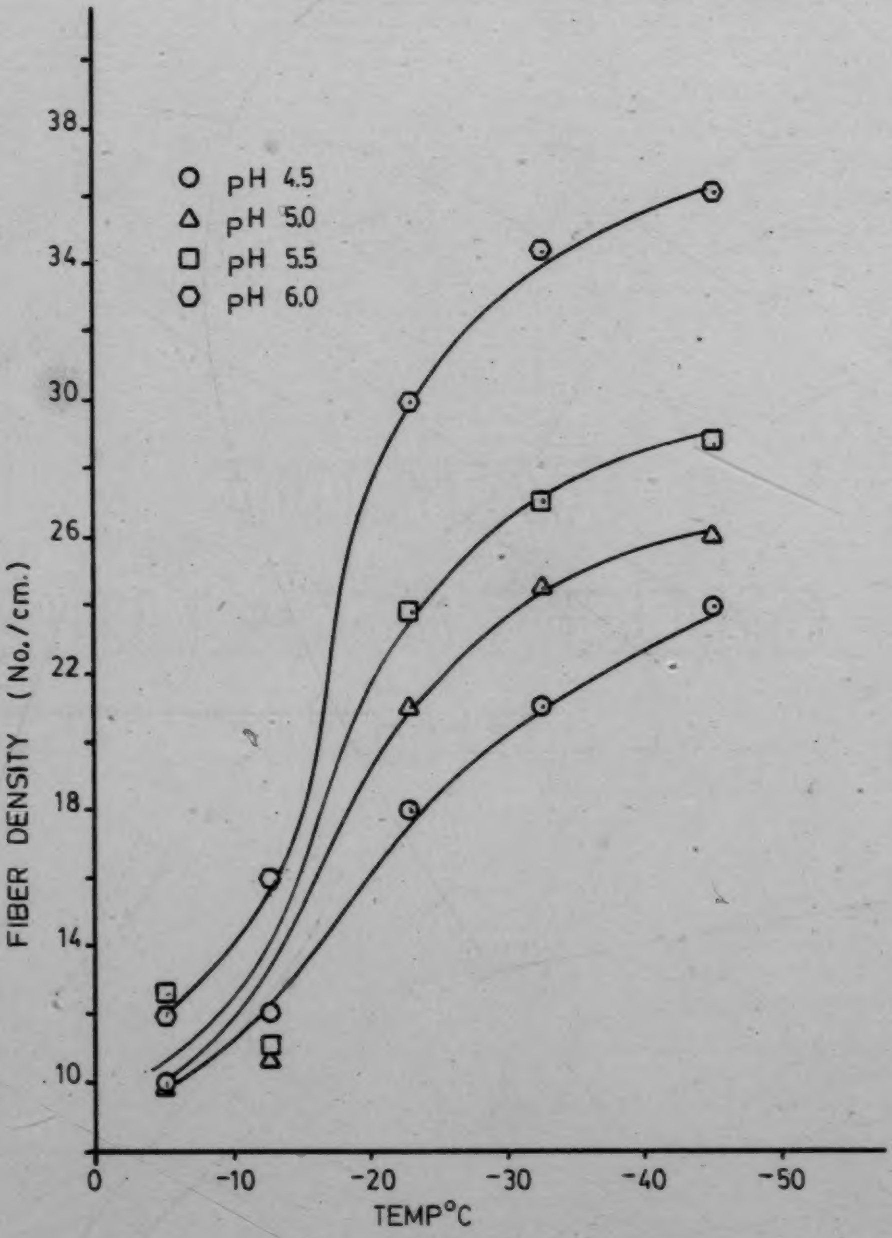

Figure 22 Effect of Freezing Temperature and Precipitation pH on Fibre Density.

to the fibre structure than at higher temperatures.

Increasing the pH of the protein to pH 6.0 increased the fibre density significantly. As shown in the texture studies in section 8.4, the change in fibre structure at different pH values is due both to the solubility effect and the effect of the initial total solids. As the pH 6.0 protein contained 10% Total Solids as compared to 12 to 14% for pH 5.0 prior to freezing, the total solids difference accounts for some of this change. Plates 13 and 14 show a decrease in fibre density with decreased total solids from 19.3% to 15.9%. Difficulties in the photographic recording did not allow a numerical evaluation of the effects of total solids on fibre density.

The fibre density values show a definite general trend, though relatively large possible errors could exist with the absolute values. The fibre density was determined as the number of fibres which intersect a unit·length. Therefore, fibres at other than a 90° angle to the line or the photographic plane will distort the value, by presenting a thicker than normal fibre. This will reduce the fibre density. Air bubbles and large voids will also cause a decrease in the fibre density. The standard deviation in the fibre density curves were within 13 to 18%, causing a significant degree of overlapping of curves. However, the general trend agrees with the textural and the final total solids analyses.

8.4 Texture

Textural measurements were run using a Kramer Shear Cell mounted in an Instron Universal Testing Instrument. The only measurement used to compare samples was the corrected peak force which had the frictional force of the cell subtracted.

Fig. 23 shows the effect of various freezing rates and the pH of the protein precipitate on the peak force. Increased freezing rates decreased the peak force at all pH's. The peak force is therefore greatest with the thicker, less numerous fibres, which are produced at slow freezing rates and low protein solubility. The thinner, more numerous fibres found with rapid freezing and high pH (pH 6.0) decrease the peak force.

The drop in peak force due to pH is attributed to both the difference in protein solubility and the concentration of water in the material. The total solids vs pH histogram shown in Part 1, Fig 5, shows that the initial total solids varied significantly with the pH. Figs. 24, 25, and 26 show the effect of equilibrating the total solids at different pH values. In all cases, increasing the initial total solids,. which produced an increase in the final total solids, caused an increase in the peak force. The pH 4.8 sample exhibited only a small increase in peak force, with increased initial and final total solids. The pH 6.2 sample exhibited the greatest. Similar initial and final total solids at different pH values, exhibited different peak forces,

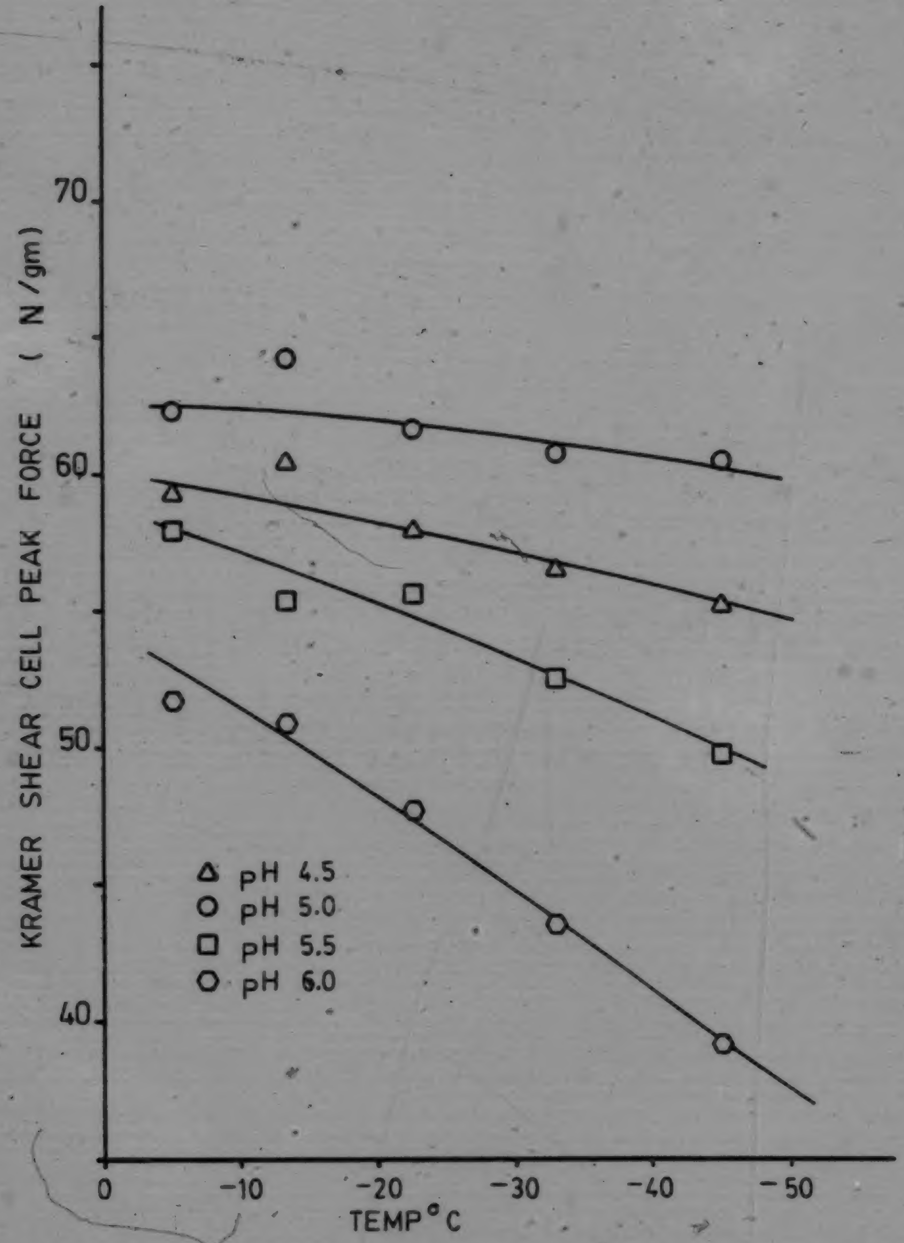

Figure 23 Effect of Freezing Temperature and Precipitation pH on Kramer Shear Cell Peak Force.

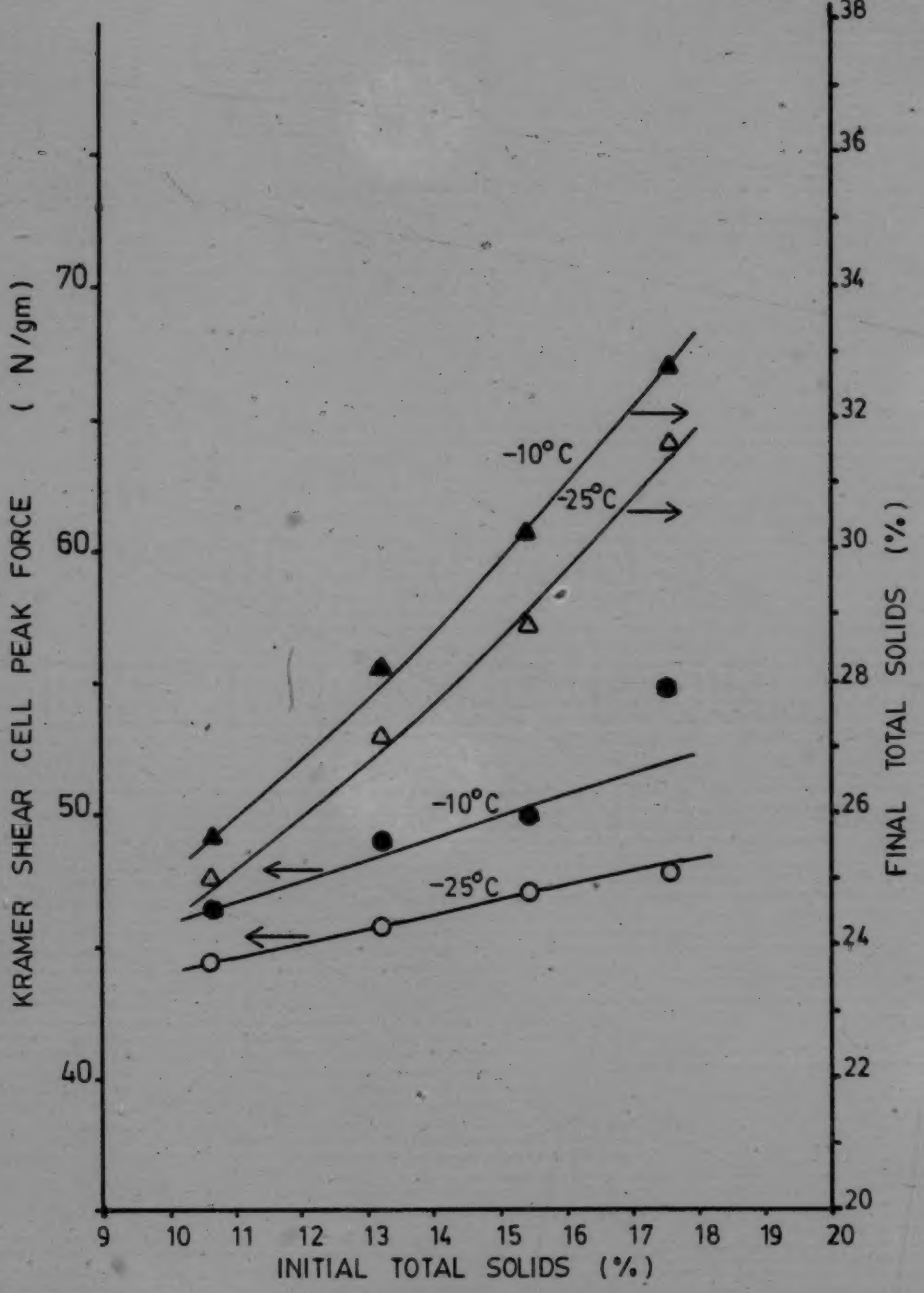

Figure 24 Effect of Initial Total Solids and Freezing Temperature on Peak Force and Final Total Solids of a pH 4.8 Precipitate.

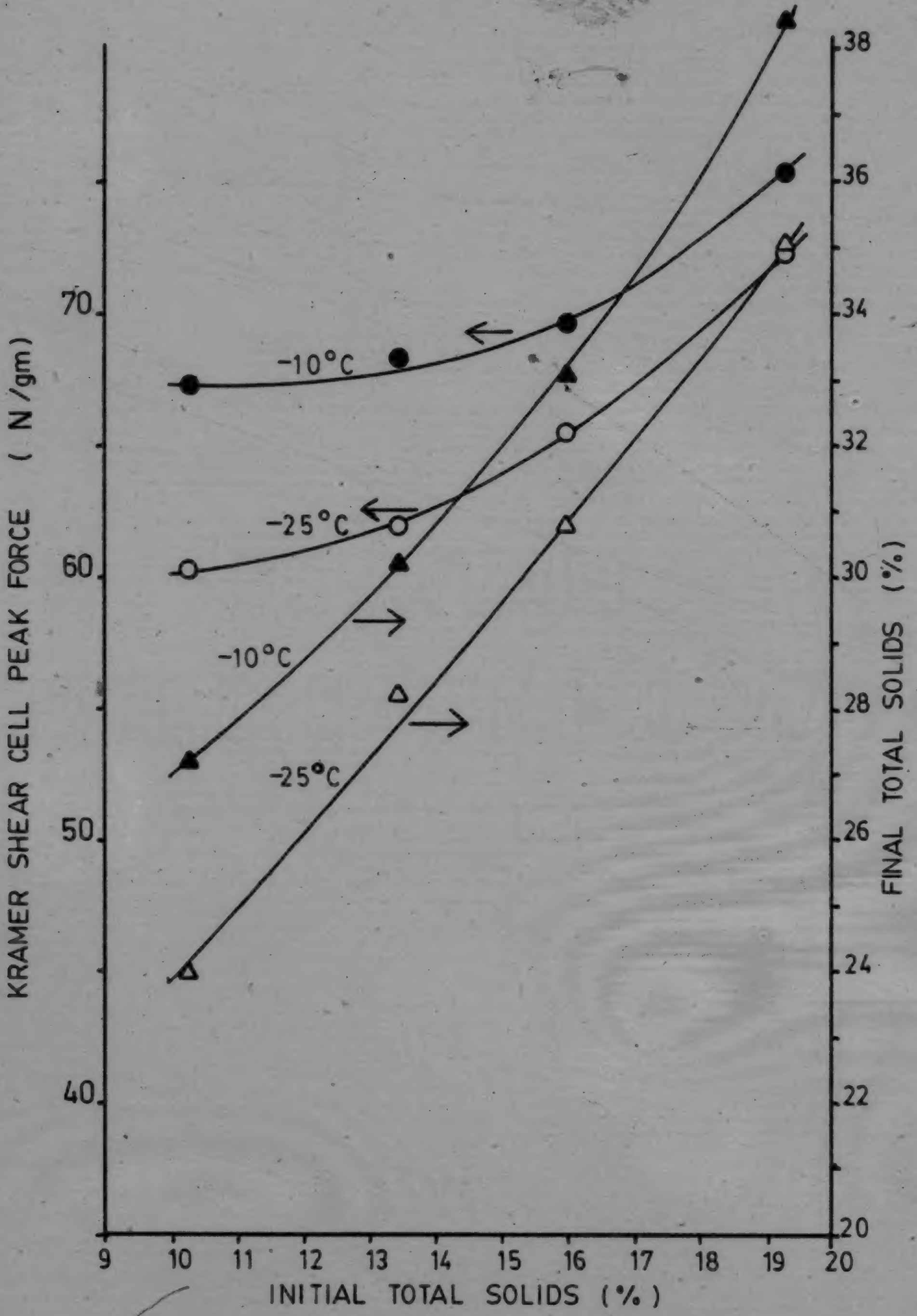

Figure 25 Effect of Initial Total Solids and Freezing Temperature on Peak Force and Final Total Solids of a pH 5.5 Precipitate.

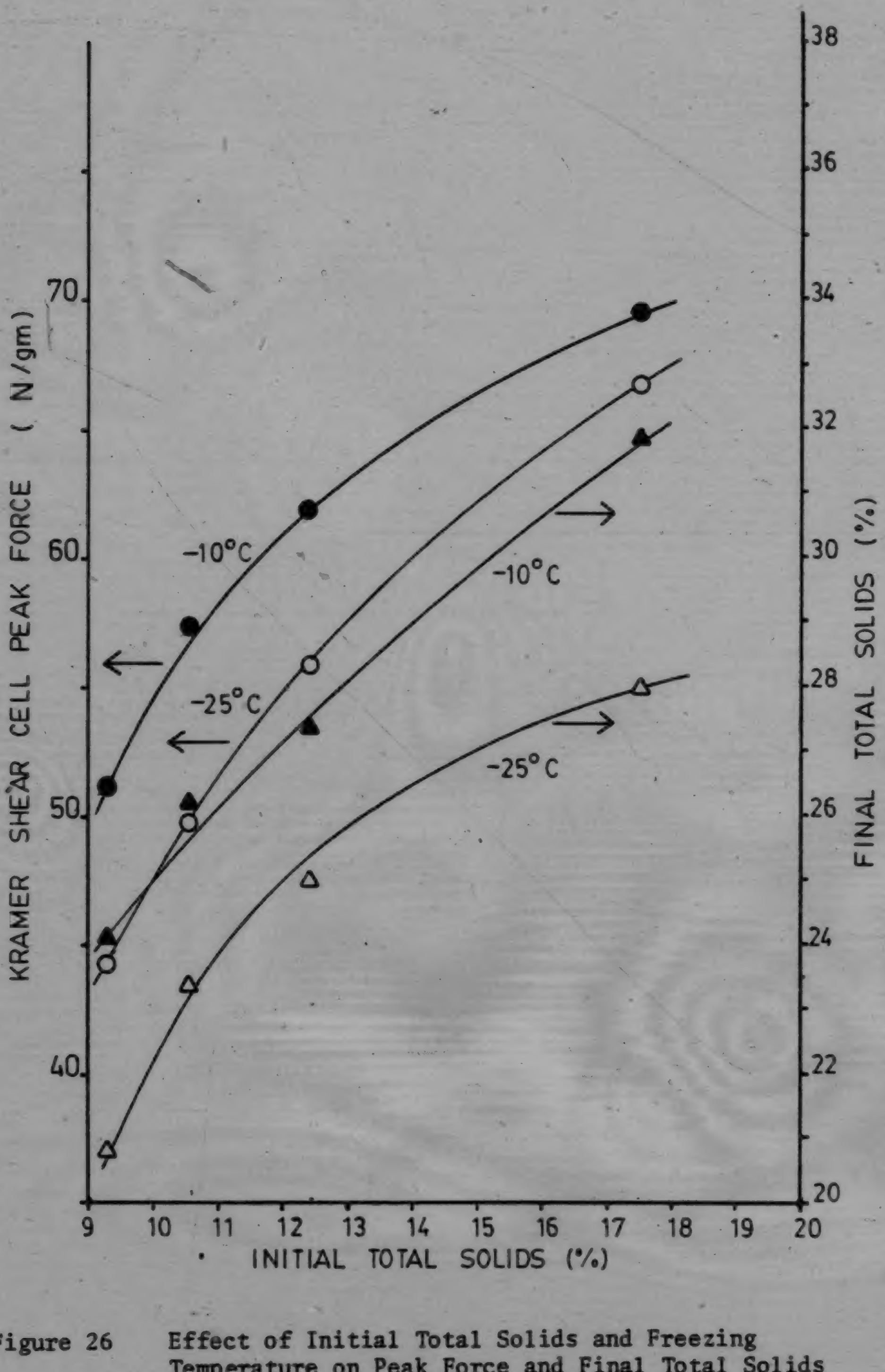

Figure 26 Effect of Initial Total Solids and Freezing Temperature on Peak Force and Final Total Solids of a pH 6.2 Precipitate.

showing the peak force to be dependent on both the pH and the total solids of the texturized product. Therefore, a substantial amount of the reduced peak force exhibited in the pH 6.0 sample shown in Figure 23 is due to a high water content.

Textural analysis of the textured samples prior to heat setting showed a slightly different trend than found in the heat set samples. A comparison of the Kramer Shear Cell peak force of the heat set to the non heat set samples is shown in Figure 27. The curves appear similar to the fibre density curves,(Fig. 22) which would suggest that the number and size of the fibres affect the change in the texture upon heat setting. The pH 6.0, fast frozen samples (-32°C) showed a greater change in the peak force than did the slowly frozen, pH 5.0 samples. The larger, more distinct fibres, with larger voids, associated with the latter case, are likely to remain intact upon thawing. The smaller, more dense fibres found at high pH, rapid freezing, are more susceptible to dissolution upon thawing, thereby reducing the strength of the non-heat set product. This dissolution of the small fibres was visibly noticeable when the samples were cut.

Samples of unfrozen, noncompressed protein precipitate which were heat set, could not be analyzed as they crumbled very easily. A frankfurter sample was used to compare the texture to a comminuted product. The peak force of 1.8 kgf/gm was lower than all of the heat set texturized protein

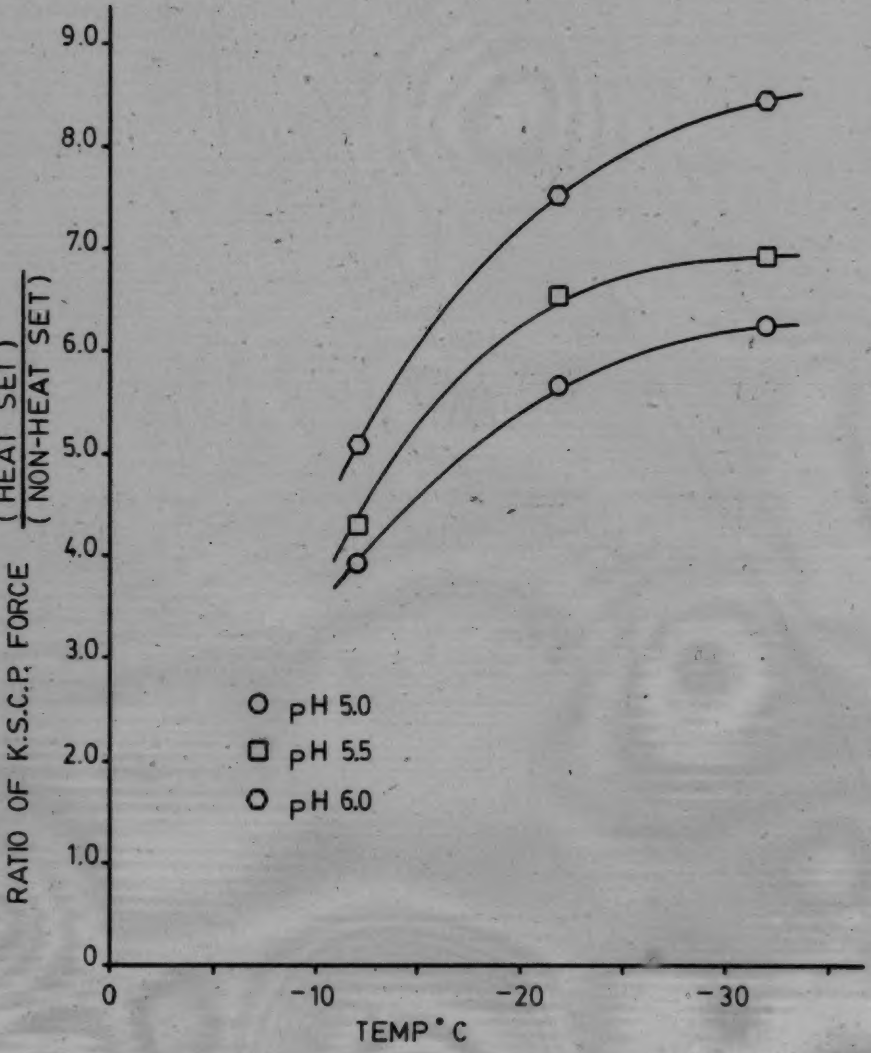

Figure 27 Effect of Freezing Temperature and Precipitation pH on the Change in Kramer Shear Cell Peak Force between Non-Heat Set and Heat Set Texturized Protein

samples.

8.5 Total Solids

The final total solids of the heat-set product was found to be dependent on the pH (and therefore, the initial total solids) and the freezing rate. Figures 24, 25, and 26 show similar rates of increase of final total solids with respect to initial total solids. The pH 6.2 sample showed the least slope, which would suggest the least drip loss after heat setting. This agrees with the photograph (Plate 3) which shows the pH 6.0 sample exhibits the least reduction in size after heat setting.

Decreasing the freezing temperature was found to decrease the final total solids of identical initial total solids samples at all pH's. (Figs. 24, 25, 26, 28). This may be due to two factors. The smaller, more numerous crystals would produce a network of fibres with a greater surface area, and a closer proximity to each other. Surface tension and capillary forces would hold a higher quantity of water in these voids than in samples with larger fibres and voids. The greater degree of crosslinking at pH 6.0 would entrap more water, especially at fast freezing rates, therefore causing the reduced drip loss.

Comparison of the different acids used for precipitation produced no significant differnce ($P<0.05$) in peak force or the final total solids.

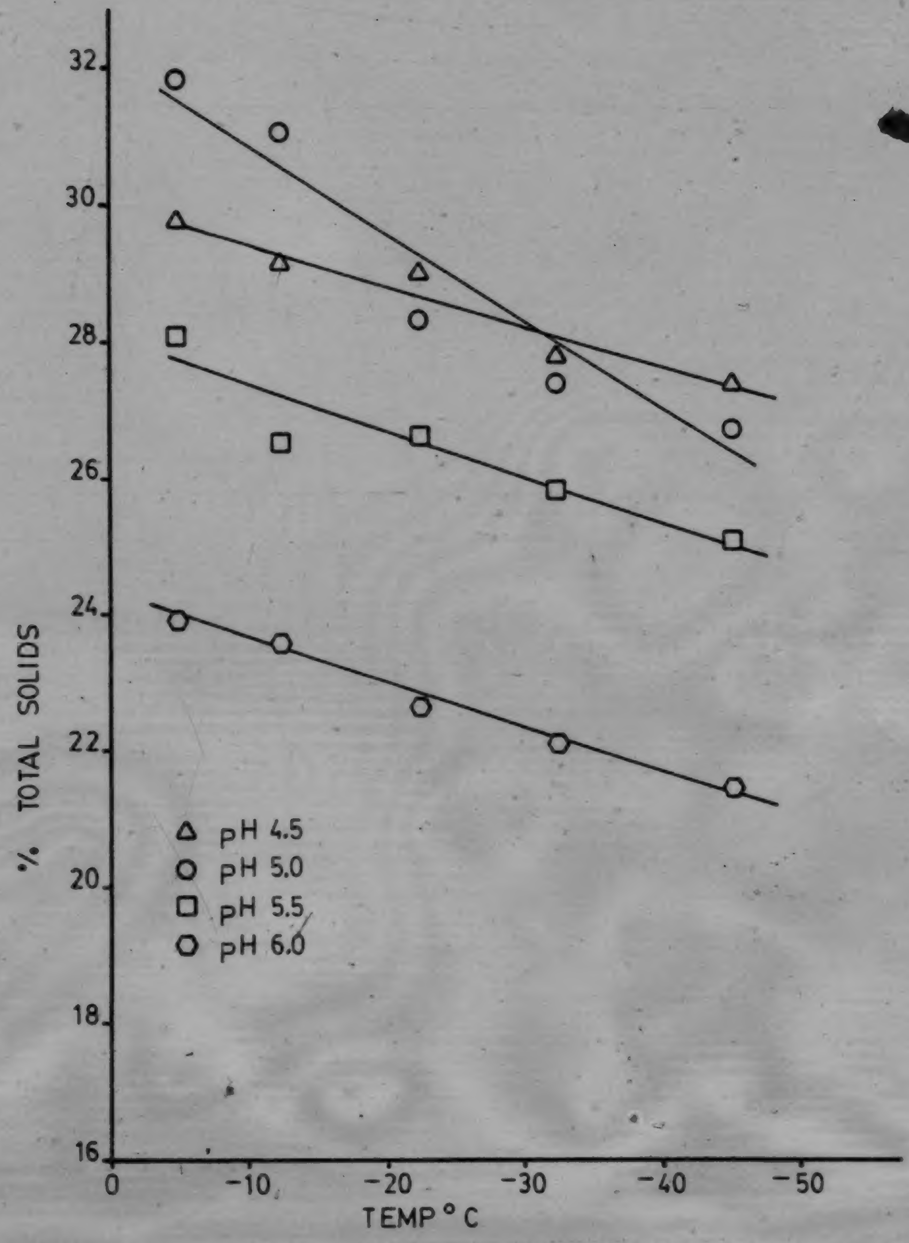

Figure 28 Effect of Freezing Temperature and Precipitation pH on Final Total Solids.

The total solids measurement is the most objective measurement of the three tests. It takes into account the voids and air bubbles and is not affected by the fibre direction as the fibre density determination was. For this reason, it is probably the best method of determining the effect of freezing rates on the fibre structure of samples with the same initial total solids and pH.

8.6 Retort Stability

Stability against structural breakdown caused by thermal treatment after the initial fibre heat setting process is mandatory for a texturized protein product which is likely to be subjected to some sort of heat treatment in secondary processing. Unexpected breakdown of the fibre structure, causing loss of texture or disintegration of the product, or a change in the toughness/tenderness of the product is undesirable.

Retorting the heat-set, freeze-texturized protein in a 2% brine solution at 121°C for 10, 20, and 60 minutes did not show any significant loss of particles from the samples into the solution. The small number of particles found in the saline solution in the retorted cans were also found in a can which contained the brine and the texturized protein, but was not retorted. This was attributed primarily to loose particles on the surface which were produced when the material was cut.

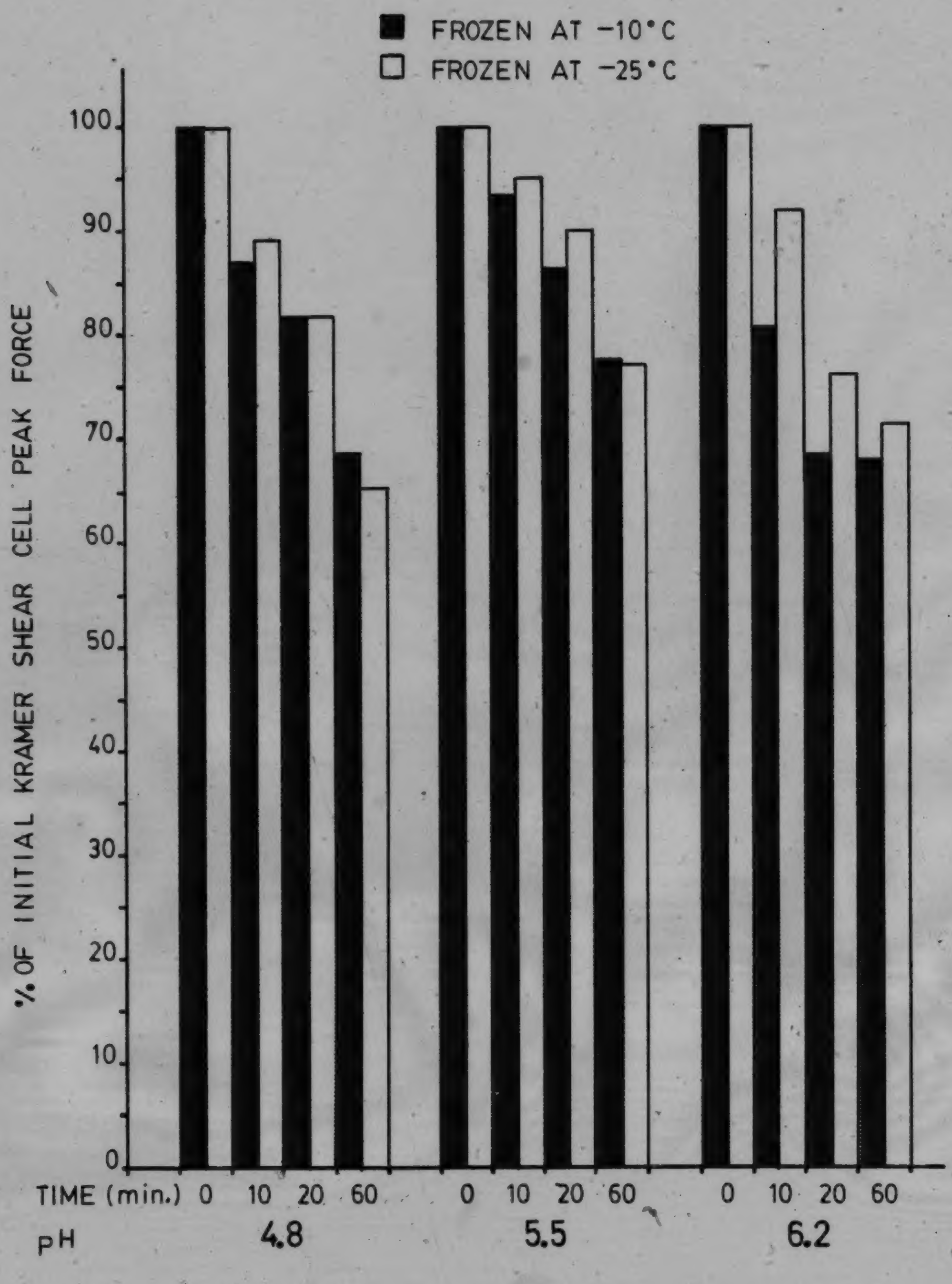

Figure 29 Retort Stability. Effect of Retort Time, Freezing Temperature and Precipitation pH on the Kramer Shear Cell Peak Force.

Fig. 29 shows a comparison of the effect of the retort time at 121°C, the pH and the freezing temperature on the Kramer Shear peak force as compared to a non-retorted sample. The initial differences in the peak forces between different pHs and freezing temperatures are accounted for, as all data were expressed relative to the appropriate non-retorted samples. Increased retort time showed a decrease in the peak force obtained from all samples. The greatest change in peak force appears to occur in the first ten to twenty minutes. The threefold increase in time from twenty to sixty minutes produced less than a threefold decrease in the peak force. This was most noticeable at pH 6.2. Without subjective testing, it cannot be established whether the reduction in maximum force correlates to a reduction in the optimal toughness/tenderness of the product. Therefore, a reduction in maximum force may not be detrimental, but actually preferred as a secondary means of modifying the textural structure.

Freezing at -10°C showed either a similar or greater reduction in peak force than freezing at -25°C. The protein precipitated at pH 6.2 exhibited the greatest effect of the freezing rates. This was more apparent at 10 and 20 minutes retort time compared to 60 minutes. The pH 5.5 precipitate showed less of an effect from the freezing rates on the peak force than either the pH 6.2 or pH 4.8 precipitate at all retort times.

The crosslinks between the fibres are suggested to be the most susceptible points to be affected by the secondary heat treatment, as the protein matrix is assumed to be essentially homogeneous. The thicker, larger fibres would show less of the effect of the secondary heat treatment due to the increased mass.

The smaller change in peak force shown at pH 5.5 could be attributed to the greater initial maximum force prior to heat treatment. Therefore an equivalent reduction in force to that found at other pHs would appear as a greater final relative force. Also, the structure, i.e. protein/protein binding, may be of a more stable form at this pH.

9. Conclusions and Recommendations

9.1 Conclusions

First generation technology for meat protein recovery is available and used in the form of mechanically deboned meats. However, in the poultry industry, this still leaves a quantity of high grade protein in the waste product. The process investigated in this research, low temperature alkaline extraction with acid precipitation, is a viable method of recovering this protein in a concentrated form, suitable for further processing.

The mild treatment offers a number of advantages over harsher treatments, and other processing methods such as mechanical deboning. The high degree of protein denaturation associated with harsher treatments such as hot rendering does not occur. Production of new amino acids, specifically lysinoalanine which may be toxic, or of reduced biological availability, was not found under these conditions. The fat content was minimal in the final product, which allows this product to be used in further processing such as texturization, which requires low fat concentrations. When added to comminuted products, this protein source will enable the addition of greater quantities of desirable surplus fats, which increases the economic value of the protein source. Furthermore, as this product consists essentially of sarcoplasmic and myofibrillar protein, with a very low collagen content, it is expected that the emulsification capacity will be high. The choice of acid used,(acetic, hydrochloric, phosphoric, or sulphuric) in the precipitation process was shown not to affect the final product, and therefore the choice will depend strictly on economic considerations. The room temperature conditions and reduced time reduces the energy costs and the cost and size of equipment required.

The bone residue after processing will be suitable, with a neutralization step, for rendering, as there is still a significant concentration of protein (collagen) in the material. The major difficulty asociated with the process is the production of a waste water "whey", containing water soluble proteins, which has to be treated in some manner. Recovery of this protein may be possible using a recycling process, or a heat coagulation step, though both of these are associated with major drawbacks.

It is suggested that this protein source would be advantageous for direct use in comminuted products due to its high protein concentration, and the fact that it is from an animal source. It was also found to be a good source material for the production of a fibrous textured product. Again it has a major advantage of being a texturized meat product, rather than a texturized vegetable protein product. This is of major concern, as texturized vegetable proteins used as meat analogs or extenders have been shown to have a poor public acceptance,(Robinson, 1972)

Textural modification processes to simulate the fibrous nature of meat are presently limited to two industrial methods, spun-fibre and extrusion. Both of these require relatively expensive equipment, and are either energy intensive or require a high degree of technical knowledge. Furthermore they subject the proteinaceous material to severe conditions.

Freeze-fibre formation is a relatively simple process utilizing present technical knowledge and equipment. Although it is also energy intensive, requiring freezing and heat setting, it does not require the high pressure, temperature environments associated with the extrusion process. However, without an extensive economic evaluation of the process, it is difficult to state whether the process would be more economical than the extrusion process, though it would definitely be expected to be more economically feasible than the spun fibre process.

The fibre structure characteristics of the freeze-texturized protein were found to be dependent on the freezing rate, the shape of the freezing mould, the nature of the freezant/protein interface and the nature of the protein material, i.e., the pH-controlled degree of solubilization, and the total solids. These structural characteristics include the size and number of fibres, the area between the fibres, the orientation of the fibres, the consistency of the fibre structure, the extent to which the fibres are bound to each other, the force exerted by a Kramer Shear Cell to "shear" the product, the ability to retain moisture (particularly in the void spaces) and the retort stability of the product. No one particular set of conditions were considered optimal as this is dependent on the fibre structure desired.

Use of a single freezant/protein interface produced a generally aligned fibrous structure, normal to the interface. The semi-infinite slab freezing mould produced a structure with a parallel fibre alignment which was the closest to simulating an unmodified muscle fibre structure. The more uniform fibre structure and associated characteristics which were found with the cylindrical mould and its radially oriented fibres are likely to be of greater importance than the high degree of parallel fibres. Furthermore, the semi-infinite slab design may prove impractical in applied processes compared to cylindrical forms.

The ability to modify the fibre structure by altering a few easily controlled parameters, ie. cooling rates, total solids, or pH, allows for a simple process control system. It also enables the production of a number of characteristically different texturized protein products. Briefly, the general trend shown was that an increased cooling rate increased the fibre density, the degree of fibre orientation, the ability to retain moisture, the retort stability and the fibre crosslinking, while slightly decreasing the Kramer Shear force. Increased initial total solids decreased the water retention and the area between the fibres, while increasing the Kramer Shear force. Increased protein solubilization (pH 6.0) increased the fibre density, the fibre crosslinking and the water retention, though slightly decreased the retort stability and the Kramer Shear force. The different fibre structures found at different pHs, were accounted for by the difference in the initial total solids, and the degree of protein solubility. No significant difference was found between samples precipitated with different acids.

Crosslinking between the fibres removed any necessity for binding agents to hold the fibres intact. The degree of crosslinking was found to increase primarily with increased protein solubilization (pH 6.0) and secondarily, with increased freezing rates, so that in certain situations (pH 5.0, slow freeze) a limited amount of crosslinking occurred. However, this lack of crosslinking may sometimes be desirable, as simulation of certain meats, such as fish, require a greater degree of fibre flaking.

The retort stability of the product was thought to be partially dependent on the size and number of crosslinks, as these would be the most probable points of initial structural degradation due to secondary heat treatments. Although there was no noticeable disintegration when the materials were retorted at 121°C for 60 minutes in a 2% brine solution, the peak force decreased by up to 35%, dependent on time and pH. The rate of reduction of peak force decreased with increased retort times. The pH 5.5

samples appeared most resistant to this degradation. The drop in the Kramer Shear peak force should not be regarded as detrimental until the subjective testing is done. It may in fact be a desirable method of tenderizing the product.

Freeze texturization was found to be a simple method of imparting a retort stable fibrous structure to a non-structured proteinaceous material. The process parameters can be easily controlled or modified to produce a desired fibre network, dependent on the intended use of the product. Although no additional components, like fats, carbohydrates, or coloring and flavoring additives were included in the material, it is suggested that some of these additives could be readily incorporated after the heat setting stage. The texturized products with the extensive crosslinking network behave in a sponge like manner which would allow for the infusion of the components into the void spaces. Possible intended uses for this texturized meat protein are as either meat extenders, or analogs. The product could be incorporated into comminuted products, used as compressed meat, ie. turkey, rolls or as small meat-like chunks for use in fresh or canned stews or soups.

9.2 Recommendations

The protein recovery section of this work still leaves several large areas uninvestigated. At present, a microbiological examination of the process is underway within the Department of Food Science, University of

Alberta. An industrial scale investigation, along with an economic evaluation of the process must be completed to determine the viability of the process. A more extensive testing of the functional properties of the protein material might also be useful.

The second half of the work was primarily an introduction to the freeze-texturization process for producing a retort stable, fibrous protein network, whose fibre structure can easily be controlled or modified.

Possible mechanisms for the fibre formation were only briefly discussed and therefore leave a large segment unresearched. This includes the effects of solute and solid concentration combined with the compaction effect and any biochemical reactions which cause protein/protein interactions at subzero temperatures. It has been suggested by other workers that freeze denaturation of the protein does not occur at the time-temperature conditions similar to those used here, though it should be confirmed for this material.

The final product will require the addition or use of other components such as fats, other protein sources or carbohydrates. Therefore it would be advisable to determine the effects of adding these components on the texture, fibre structure, uniformity, etc. of the product, when added prior to freezing or after heat setting. As increased viscosity has been shown to inhibit crystal growth in such products as kori-tofu, the analysis of the effects of viscosity, through the addition of gelling agents, should be investigated. Sensory analysis of this product in comminuted products, as meat rolls, or as small meat patties, etc., would help to determine possible uses. Finally, an economical feasibility study should be iniated to compare this process and the resulting products to presently used industrial texturization processes.

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